Controlling *Fusarium* species and their mycotoxins in cereals

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1. Introduction

"Freedom from hunger is not only a basic human right: it is essential for the full enjoyment of other rights, such as health, education and work, and everything that flows from these." [1]

1.1. Worldwide cereal production

Agriculture is essential for human beings because its products feed the global human population. The demand for food is increasing along with the size of the human population; the global human population is estimated to increase to 9.7 billion in 2050 [1]. Many agricultural plants serve as important food to humans and livestock, but cereals are among the key crops, with 3.75 billion tons of production per year worldwide [2]. Cereal production includes wheat, rice, corn, oat, barley as well as many other species. Wheat in the genus *Triticum* is actually the world' s major cereal crop [3] (Fig. 1). Although diversity in cereal production is strong in Europe and Canada, wheat, barley and corn predominate in human and animal food industry. Wheat is on the top of the most needed raw materials in industry, with 25% of the global production area in Europe [4] and with 137, 9 million tons of production in 2018 [5]. From one of its rawer forms, the flour, important products such as bread, pasta, semolina, couscous and cakes are made. Furthermore, flour is an important compound of higher-level processed food.

World wheat market

	2015/16	2016/17	2017/18	2018/19 estimate	2019/20 forecast	
					Previous (05 Sept 2019)	Current (03 Oct 2019)
	(million tonnes)					
Production1/	736.7	761.3	759.6	730.4	766.9	**766.0**
Supply2/	965.2	1 004.0	1 023.2	1 014.4	1 034.6	**1 034.8**
Utilization	716.7	736.4	737.6	746.7	760.1	**761.5**
Trade3/	167.1	176.8	177.0	167.8	173.2	**173.5**
Ending Stocks4/	242.7	263.6	284.0	268.8	273.6	**272.9**
	(percent)					
World stock-to-use ratio	33.0	35.7	38.0	35.3	35.5	**35.4**
Major exporters' stock-to-disappearance ratio5/	18.0	19.8	21.0	17.5	16.4	**16.4**

Figure 1: World wheat production in million tons (FAO)

1.2. Wheat Production

1.2.1. Origin and varieties of wheat

Wheat belongs to the family of *Poaceae*, the grass family. Emergence of wheat happened 30 000 years ago in the mountains of Karaca Dag in Turkey [6]. *Triticum uratu* is the oldest common progenitor that is known. After natural hybridization of *Triticum uratu* with *Aegilops speltoides* (also of the grass family) and a single mutation conferring a stronger rachis to keep grains on the ear, about 7000 years ago, the actual variety *Triticum durum* was born, also called winter wheat [6] and is used for pasta production. *Triticum durum* is – unlike its two parental species – tetraploid. A second hybridization event between winter wheat and *Aegilops tauschii* gave rise to *Triticum aestivum*, also known as spring wheat [6] and used for bread production. *Triticum aestivum* is hexaploid. The evolution of wheat is illustrated in Fig. 2 [7]. Nowadays, winter wheat and spring wheat are varieties that are cultivated around the world. Winter wheat is sown in fall, lives through the winter in the vegetative state and is then harvested in the summer. Spring wheat is sown in spring and is harvested in fall. Winter wheat is harder and usually contains a higher protein content than spring wheat.

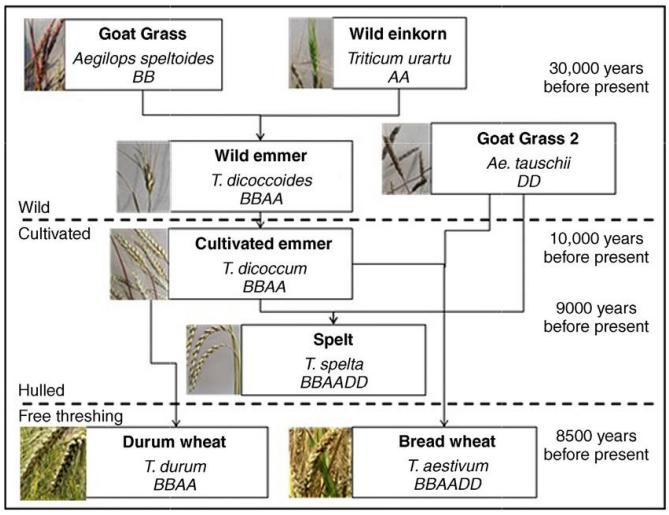

Figure 2: Evolutionary relationship of wheat and its ancestors (adapted from New Hall Mill, Tadesse et al. 2016)

1.2.2. Cultivation of wheat and agronomic practices

Improvement of agronomic practice is strongly dependent on the developmental stage of the culture. The Zadoks scale (Fig. 3) is a cereal development scale that was proposed by the Dutch phytopathologist Jan C. Zadoks and is now widely used in cereal research and agriculture ("Biologische

Bundesanstalt, Bundessortenamt und CHemische Industrie", BBCH). This scale was used in this work for the wheat assays.

As mentioned above, the sowing of winter wheat is done in fall and that of spring wheat in spring. The different stages of development are the same for both species, although winter wheat needs a cold period for heading. Leaves appear one after the other and after BBCH 20, tilling starts [8]. At BBCH 41, the flag leaf is completely extended and the stem begins to elongate until BBCH 63 at full flowering. The appearance of the ear and flowering time follow after about 8 days, and all the biomass production is then concentrated on grain development and filling. At BBCH 89, grains are full and mature, plants senesce and they are ready to be harvested.

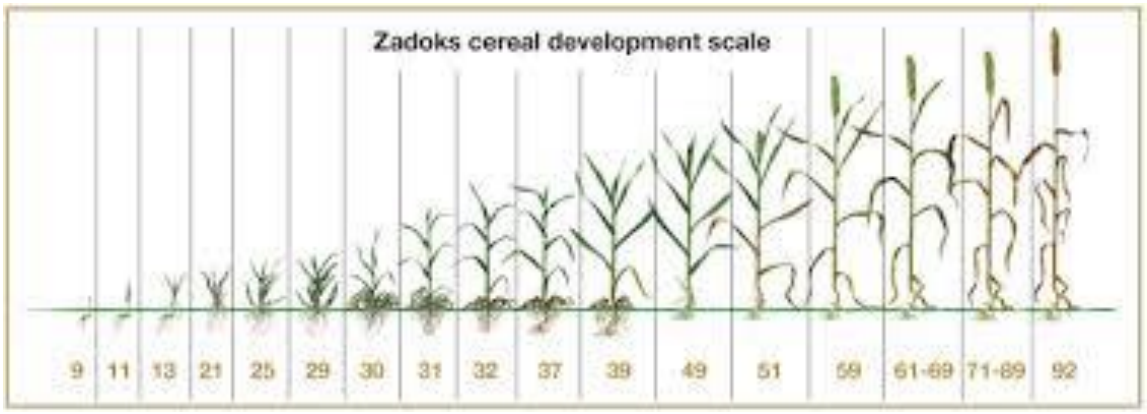

Figure 3: Growth stages on wheat (usda.gov)

Increasing and securing wheat yield per unit area can be achieved by breeding high yielding varieties and applying the optimum cultural practices to sustain soil fertility, through their effect on the physical, chemical and biological properties of the soil [9]. Fertilization is essential during the entire life because of high need of access to potassium and nitrogen influences root growth, tilling, foliar growth and grain filling. Furthermore, agronomic practice also includes plant protection against pests and weed management.

Wheat grains have a typical anatomy that distinguishes them from naked grains (Fig. 4). The germ contains the embryo, the future plant, and the kernel-endosperm. The endosperm confers 80% of the total weight of the grain and contains mainly starch and some protein [6]. Winter wheat has a very hard kernel, created by proteins called gluten. A higher gluten content in flour of winter wheat leads to more resistant and less extendible doughs, compared to spring wheat. However, total protein content is identical between winter and spring wheat (12-15%), and grains of winter wheat have more carotenoids than spring wheat but fewer peroxidases [10]. After harvest, the thousand grain weight

(TGW) is measured to determine the protein amount of thousand grains, which is an important factor for industry.

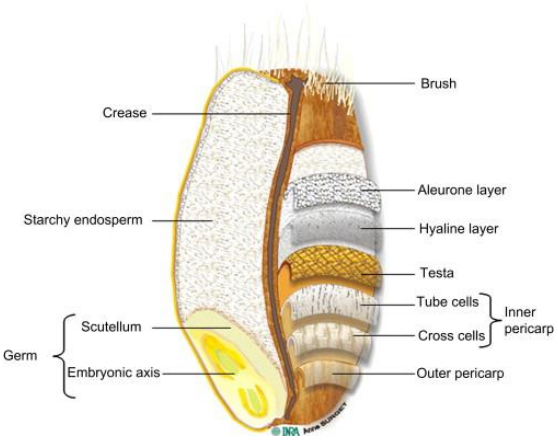

Figure 4: Anatomy of a wheat grain (sciencedirect.com)

1.2.3. Challenges in wheat production

It is of high importance that both yield and quality of the grains are consistently high. Food industry works in close collaboration with research institutes, which constantly check and ensure safety of products and guarantee food quality.

Buyers have several requirements for grain quality. A good grain quality and a high thousand grain weight allow better transformation processes, and finished products are more resistant to heat. Furthermore, proteins in the grains are responsible for the culinary quality of the products. For example, in pasta industry, winter wheat is preferred over other cereals due to its organoleptic properties and fabrication. Therefore, across the world, winter wheat is mostly used by the pasta industry; pasta consumption is responsible for the use of up to 70% of winter wheat [6]. Spring wheat is mostly used in bakery, where several criteria have to be fulfilled to have a good flour quality: good hydration capacity, good stability during the raising of the dough and a protein content above 11% [11]. Additional aspects of quality exist; for example, endosperm texture affects wheat milling, the performance of wheat during milling and the resultant flour quality [12].

Farmers focus on similar quality criteria. First, health quality standards of the grains and standards of minimal microbial contamination have to be met. Second, the yield has to be as high as possible. Finally, minimal organoleptic quality standards are required. The norm ISO11051 [13] defines these quality criteria. If one of them is compromised, the price for grain is reduced (current price is about 166-180€/t [14]). Health quality standards of the grains are not met in the following situations:

- Heavy metal such as cadmium (0.2mg/kg [6]) present in the soil

- Pesticide residues
- Mycotoxin content produced by fungal pathogens

Global climate changes and increasing food demand due to a worldwide growing human population necessitate improving grain quantity while respecting quality standards and standards for none-diseased grains. The latter two are the most important factors that reduce the amount of grain biomass that goes into food industry.

1.2.4. Diseases of wheat

Figure 5: FHB disease (J. Mark)

Wheat is regularly affected by herbivory attacks from insects and virus (facilitated by nematode and insect vectors), but fungal attacks remain very important and are the most common negative biotic factors in wheat. A number of fungal diseases are common: several seed diseases, Septoria (*Zymoseptoria tritici, Zymoseptoria passerinii*), rust (*Puccinia triticina*) and mildew. Diseases with the highest impact on grain quality are Ergot (*Claviceps purpurea*) and Fusarium head blight (FHB); they are among the most threatening cereal diseases because of their capacity for mycotoxin production (Fig. 5). The importance of each of the fungal diseases mentioned also depends on the susceptibility of the wheat variety.

1.3. Fusarium head blight (FHB)

1.3.1. FHB complex

Fusarium head blight causes severe damage in wheat cultivation. Yield loss per year was estimated between 20 and 50% in Argentina and more than 1 million tons in China [15], and prognostics are estimated every year for farmers [16]. Over 17 *Fusarium* species associated with FHB have been isolated from naturally infected spikes [17]. Among the most abundant are *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. tricinctum*, *F. sporotrichioides*, *F. langsethiae*, *F. proliferatum*, *F. verticillioides*, *F. cerealis*, *F. subglutinans*, *F. poae*, *F. acuminatum*, *F. equiseti and F. cerealis [18, 19]. Microdochium* sp. such as *M. nivale* and *M. majus* are part of the FHB complex [20]. FHB has common symptoms and causes brown, dark, purple to black necrotic lesions on the exterior surface of the florets and glume [21].

Fusarium species differ in their geographic distribution (Table 1), and a good indicator of distribution is temperature. Accordingly, the pool of species differs among warmer and cooler areas. *F. graminearum* is most common in moist and warm climates of southern Europe, whereas *F. avenaceum* and *F. culmorum* are found in European countries with a cool climate. *F. poae*, *F. tricinctum*, *F. cerealis*, *F. sporotrichioides* and *F. equiseti* are most common in countries of central and northern Europe [22]. *F. graminearum* is the most frequent and the most virulent species [18], while *F. culmorum* and *F. poae* are reported to increase in some European countries [23].

Table 1: Fusarium *species in European countries*

Dominant species	Country
F. graminearum, F. culmorum, F. verticillioides, F. equiseti	*Switzerland [24, 25]*
F. graminearum, F. culmorum, F. poae, F. avenaceum, F. verticillioides, F. equiseti	*France[24, 26, 27]*
F. graminearum, F. culmorum, F. poae, F. cerealis, F. tricinctum, F. cerealis	*Germany[24]*
F. graminearum, F. culmorum, F. poae, F. cerealis, F. tricinctum, F. cerealis	*Czechia[24]*
F. graminearum, F. culmorum, F. cerealis, F. avenaceum, F. poae	*Italy [21]*
F. graminearum, F. avenaceum, F. culmorum, F. tricinctum	*Sweden [18]*
F. poae, F. tricinctum, F. culmorum, F. graminearum	*Poland[24]*
F. equiseti, F. poae, F. sporotrichioides	*Hungary[24]*

1.3.2. Life cycle of *Fusarium*

Fusarium spp. have both sexual and asexual life cycles [28]. Both life cycles lead to airborne spores which infect floral tissues (Fig. 6) and contaminate grains with mycotoxins. The asexual life cycle includes mitotic sporulation (multiplication of an identical spore without fusion). Three types of mitotic spores are distinguished: microconidia produced from conidiophores, macroconidia (Fig. 7) produced from sporodochium and chlamydospores (overwinter survival form) produced from hyphae. Microconidia and macroconidia can colonize the host, while chlamydospores develop into perithecia to restart the cycle when the conditions are optimal (sexual life cycle). Chlamydospores germinate and grow as haploid mycelium. After meiosis development ends in a perithecium containing asci with spores. Flowering timing of cereals is the most critical period for infection by FHB disease as spores get into the flowering ears. During anthesis, the anthers naturally split to release pollen, which provides openings for the pathogen to get into the plant [29]. The infection results in dark brownish lesions on ears, similar to dark spotting. Lesions lead to shriveled kernels, a decrease in grain weight and reduced

yield. The fungus attacks the pericarp and penetrates cell walls to enter the endosperm and then digests storage proteins and starch [10]. The germination rate and seedling vigor are reduced when seeds are infected [23]. Temperatures between 15 and 25 °C and the alteration between wet (a_w=0.98) and dry (a_w<0.90) periods are particularly favorable to floral infection by FHB [30]. *Fusarium* is able to colonize ears from spikelet to spikelet, and the infection spreads vertically up and down the rachis axis and laterally from spikelet to spikelet (Fig. 8). Infection accumulates at the surface of the rachis/spikelet before producing aerial mycelium with macrospores, and a new cycle is able to start [31].

F. graminearum spores germinate within 6-12h of plant contact and mycelia grow without any visual symptoms on the surface of floral tissues [15]. After 48-72h, infection reaches subcuticular tissues [32]. More than 10 000 genes were detected in *F. graminearum* associated with the infection and involved genes in cell wall biogenesis and degradation, protein processing, lipid metabolism, signaling and secondary metabolism (mycotoxin production) [33]. Moreover, it was suggested that *F. graminearum* uses host-specific gene expression to modulate its primary response. In early stages of infection, terpenoid synthase (TS), trichodiene synthase (TRI5) and culmorin biosynthesis are expressed to produce mycotoxins [32]. Mycotoxin content is dependent on incubation temperatures, with an optimum between 22-28°C, and on time [34].

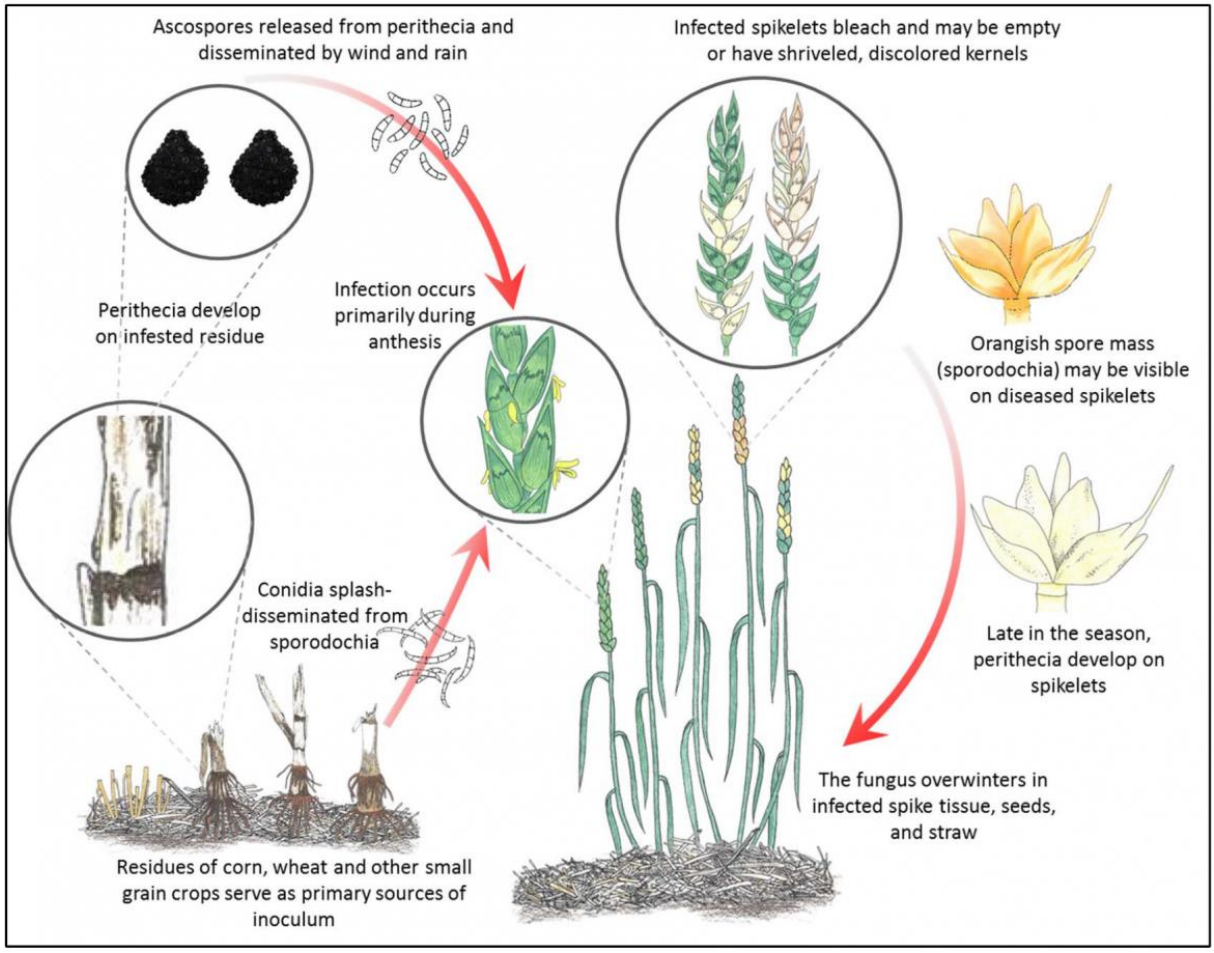

Figure 6: Life cycle of Fusarium *(Agsolution.ca)*

Figure 7: Macroconidia of F. graminearum. *Scale bar = 25µm (Leslie et al., 2003)*

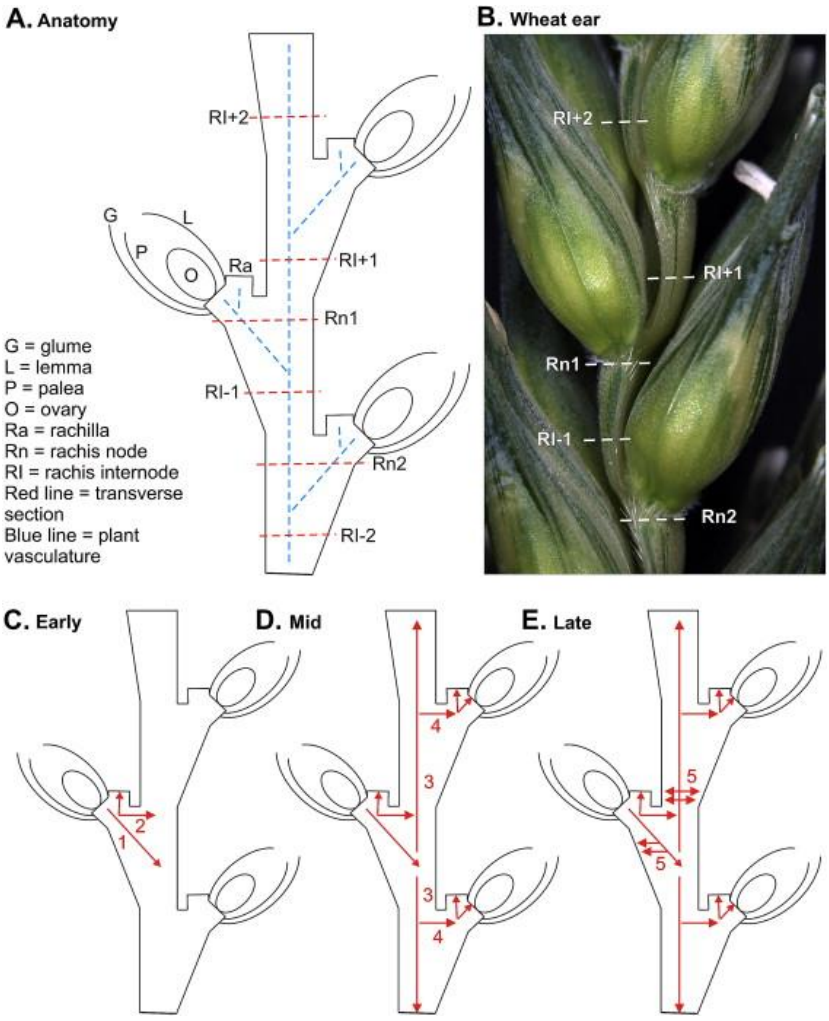

Figure 8: Way of infection of F. graminearum *in the ear (Brown et al., 2010)*

1.3.3. Species concepts and how to define a *Fusarium* species

Fusarium species are mainly distinguished based on trait discontinuities or genetic discontinuities, best in line with the morphological species concept and the genetic species concept, respectively. Consequently, there are several methods to identify the species of a *Fusarium* isolate. The method may also depend on further aspects: the host from which the isolate originates, and the degree of resolution required in the identification. Three main methods of species identification exist:

- Macroscopic and microscopic identification (Fig. 9): description of the plant disease and the symptoms observed on the diseased plant. The conditions (humidity, temperatures) have to be described under which the disease appeared [35]. The fungus is isolated, purified from contaminations, and microscopic analysis is done. The isolate is grown on different media,

which allows to evaluate different developmental states such as pigmentation, spore production, spore shape, size of macroconidia and microconidia [36].

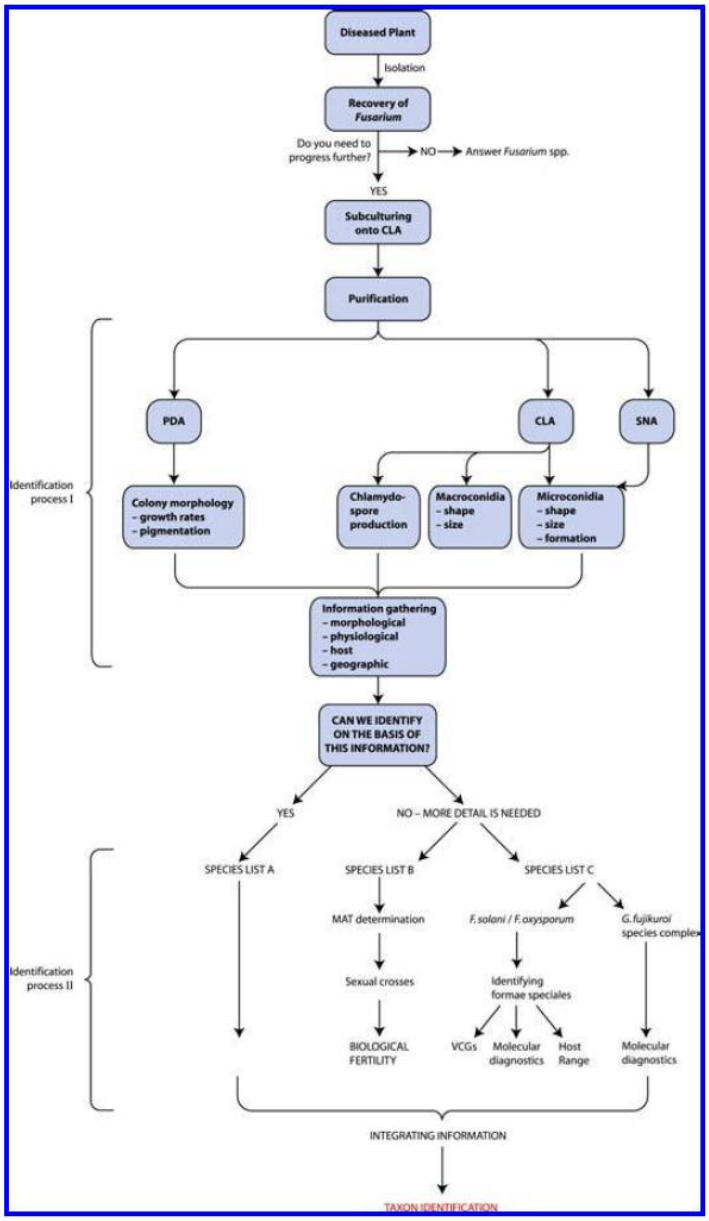

Figure 9: Protocol of macroscopic and microscopic identification (Leslie et al., 2003)

- Molecular identification: The markers of choice for species-level phylogenetic analyses in fungi are intron-rich parts of protein-coding genes [37]. The following are commonly used for species identification:

- *TRANSLATION ELONGATION FACTOR 1- α* (*TEF 1-α*) [38]
- *CYTOCHROME P 51C* (*CYP51C*) [39]
- *INTERNAL TRANSCRIBED SPACER* (ITS) [40] using primers ITS1F and LR6

The usual procedure for molecular identification is outlined in Fig. 10.

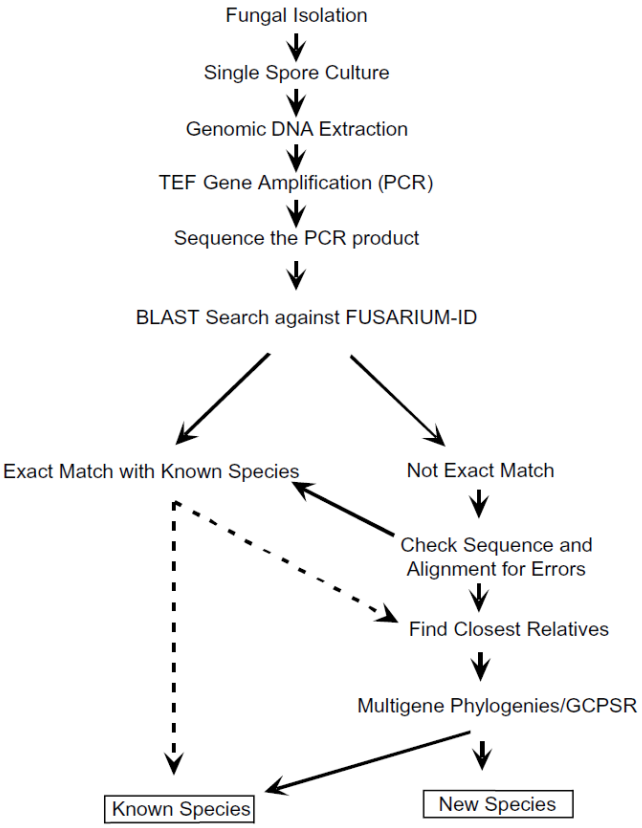

Figure 10: Molecular identification of Fusarium *species (Geiser* et al.*, 2004)*

- Further molecular tools to identify causal agents and to quantify amounts in plant tissues: A number of additional molecular techniques are being utilized in order to investigate the diversity of the pathogens responsible for FHB. The majority of techniques are based on DNA or RNA analysis [41] or high-pressure liquid chromatography (HPLC) by measuring ergosterol quantity. Polymerase chain reaction (PCR) is a sensitive and potentially specific tool to detect, identify and quantify species (fungal biomass) present within a plant. PCR assays are available for *F. graminearum*, *F. culmorum*, *M. nivale*, *F. majus*, *F. poae*, *F. cerealis*, *F. avenaceum*, *F. verticillioides*, *F. sporotrichioides*, *F. langsethiae*, *F. equiseti*, *F.tricinctum*, *F. subglutinans* and *F. proliferatum* [42] [43].

1.3.4. Host specificity of *Fusarium*

Fusarium sp. is not only a disease on cereal but can also infect other crop species. Most of the species are host-specific (table 2), although there are some which can infect several hosts such as *F. oxysporum* with its hosts of banana (Fig. 11) and humans, and *F. solani* with its hosts of plants and humans.

Fusarium infections on humans mainly affects immunocompromised patients [44] and cause allergic diseases and mycotoxicosis.

Table 2: Host specificity of Fusarium *sp.*

***Fusarium* species**	**Host**
Fusarium oxysporum	Banana, tomato, vanilla [35], human [44]
Fusarium manginifera	Mango [35]
Fusarium fujikoroi	Rice[35]
Fusarium solani	*Aglaonema commutatum* [35], human [44]
Fusarium graminearum	Barley, corn, wheat [24]
Fusarium culmorum	Barley, corn, wheat [24]
Fusarium poae	Wheat ref[24]
Fusarium sporotrichioides	Wheat [24]
Fusarium langsethiae	Barley, wheat [45]
Fusarium tricinctum	Barley, wheat [46]
Fusarium avenaceum	Barley, Wheat [24]
Fusarium equiseti	Wheat [46]
Fusarium verticillioides	Corn [46]
Fusarium proliferatum	Corn [24]
Fusarium subglutinans	Corn [24]
Fusarium acuminatum	Barley [46]
Fusarium cerealis	Wheat, corn [24]

Fig. 1. Typical disease symptoms caused by various species of *Fusarium*. A, Fusarium wilt of banana caused by *F. oxysporum* f. sp. *cubense*. B, Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*. C, Stem rot of vanilla caused by *F. oxysporum*. D, Mango malformation caused by *F. manginifera*. E, Fusarium wilt of Canary Island date palm caused by *F. oxysporum* f. sp. *canariensis*. F, Bakanae disease of rice caused by *Fusarium fujikuroi*. G, Stalk rot of sorghum caused by *F. thapsinum*. H, Root rot of *Aglaonema commutatum* caused by *Fusarium solani*. I, Cob rot of maize caused by *F. verticillioides*. All photos by authors except D, by Randy Ploetz, E, by Suzanne Bullock, and G, by Larry Claflin.

Figure 11: Fusarium *disease on different crops (Summerell et al., 2003)*

1.3.5. Mycotoxins produced by FHB

The genus *Fusarium* contains important mycotoxin-producing species that have been implicated in human diseases such as alimentary toxic aleukia, Kashin-Beck disease, Akakabi-byo or scabby grain intoxication and esophageal cancer. Also, mycotoxin-related animal disease are known: hemorrhagic, estrogenic, emetic and feed refusal syndromes or fescue foot [47-50]. Mycotoxins accumulate in agricultural products during the culture or during storage. Another important aspect of mycotoxin production is that each species has its own panel of mycotoxins and that complicates toxin detection [22] (Table 3). The most common mycotoxins are those of the trichothecene family, with deoxynivalenol (DON, also known as vomitoxin), nivalenol (NIV) and T2/HT2 [51]. But also other mycotoxin families are of high interest, including fumonisin, zearalenone, beauvericine, eniatines and moniliformine [52].

Table 3: Mycotoxin production of each species of the FHB complex

Mycotoxin	***Fusarium* species**
DON	*F. graminearum, F. culmorum* [24]
NIV	*F. graminearum, F. culmorum, F. poae, F. cerealis, F.equiseti* [24] [53]
T2/HT2	*F. sporotrichioides, F. langsethiae, F. acuminatum, F. poae* [24, 53]
Fumonisin	*F. verticillioides, F. graminearum, F.proliferatum, F. subglitinans* [24, 51, 54]
Zearalenone	*F. graminearum, F. culmorum , F, equiseti [22] , F. cerealis* [24]
Beauvericine	*F. sporotrichioides, F. langsethiae, F. poae, F.proliferatum, F. subglutinans, F. avenaceum* [24, 53, 55]
Eniatine	*F. tricinctum, F. poae, F. avenaceum* [23, 55, 56]
Moniliformine	*F. tricinctum, F. avenaceum, F. subglutinans, F. proliferatum* [24]

Trichothecene are potent inhibitors of protein translation in eukaryotes [57, 58] and more than 200 trichothecene have been reported [59]. They all have different specific effects, and DON can contribute to the virulence of *F. graminearum* during the infection of wheat [60]. This explains why very often toxins are produced before any visual symptom are visible on the plant. Despite the fact that in the past 20 years the trichothecene biosynthetic pathway has been described [61], the control

of trichothecene remains difficult, and therefore the European Commission set legislative limits for *Fusarium* mycotoxins (Table 4) [62].

Table 4: Typical EU mycotoxin maximum limits (ML) and toxicity (EURL)

	Lowest* ML µg kg^{-1}	Typical ML µg kg^{-1}	highest ML µg kg^{-1}	Typical toxicity		
Aflatoxin B_1	0.10	Certain cereals, cereal products, dried fruit, peanuts, tree nuts	2.0	Almonds, pistachios and apricot kernels	8.0	Potent mutagens genotoxic carcinogens (liver cancer)
Sum of aflatoxins B_1, B_2, G_1,G_2	-		4.0		10.0	
Aflatoxin M_1	0.025	-	-	-	-	
Ochratoxin A	0.50	Cereals	3.0	Liquorice extract	80	Teratogenic, causes liver and kidney damage
Patulin	10.0	Apple compote, or puree	25	Fruit juices	50	Embryotoxic genotoxic mutagen
Deoxynivalenol	200	Bread	500	Unprocessed maize, durum wheat & oats	1750	Acute GI symptoms, e.g. vomiting and bloody diarrhoea
Zearalenone	20	Bread	50	Refined maize oil	400	Not acutely toxic, but oestrogenic
Fumonisins	200	Maize breakfast cereals & snack	800	Unprocessed maize	4000	Possibly carcinogenic, (oesophageal cancer) and implicated in neural tube defects
T-2 and HT-2 toxin§	15	Breakfast cereals	75	Oats (with husk)	1000	Organ haemorrhage, oral lesions, dermatitis, leucopenia, blurred and painful vision

* in food for infants and young children § Note: currently recommendation only

1.4. Control of FHB

1.4.1. Methods of control

There are several approaches for FHB management. Among them, there is the control during wheat cultivation, which is based on avoiding or limiting the exposure of cereal spikes to spores during flowering [17, 63]. Interventions include crop rotation with planting crops which are not a host of *Fusarium* species [64], tillage operations that bury infested cereal residues, or burning the residues [65]. Appropriate use of fertilizers and seed treatment are also possible control options against FHB. Biological control with bio-control agents including bacteria and fungi [28] can help reduce *Fusarium* infection. *Brevibacillus*, *Streptomyces*, *Trichoderma gamsii* are recommended for testing as potential FHB bio-control agents. Genetic control includes host plant resistance and breeding for resistance quantitative trait loci (QTLs). There are 52 QTLs known to confer FHB resistance [28]. A recent study showed an RNAi-based control of *F. graminearum* by spraying dsRNAs on plants [66]. The same approach was also tried in other fungal pathogens, but it seems not particularly effective and still needs to be improved [67]. Chemical control is the single most effective control tool for FHB (30 to 40 % of

efficacy). Fungicides have been used for more than four decades against FHB, and several studies have tested the efficacy (see below).

The routine use of fungicides to control crop diseases has been an important element in the progresssion of modern agriculture and helped increase crop yield, improve quality and ensure stability of production. Fungicides disrupt particular cellular processes and bind to specific protein targets [68]. Since the mid-1970s, the systematic use of fungicides on cereal crops has established in Europe, especially on wheat and barley (Table 5). First, methy- benzimidazole carbamates (MBCs) were introduced, which marked the start of foliar fungicide application and increase of crop yield. The introduction of MBCs was followed by that of demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs) in the 1990s, and from 2002 onward, succinate dehydrogenase inhibitors became available (SDHIs) [68].

Table 5: Inhibitors used against FHB

Fungicide class	Cellular function affected	Target potein
Methyl benzimidazoles (MBCs)	Cytoskeleton	B-tubulin
Demethylation inhibitors (DMIs)	Membrane biosynthesis	Sterol 14α-demethylase (*CYP51*)
Quinone outside inhibitors (QoIs)	Respiration	Mitochondrial cytochrome b
Succinate dehydrogenase inhibitors (SDHIs)	Respiration	Succinate dehydrogenase

The severity of FHB disease is predicted to increase due to climate change and its control is problematical due to the difficulty of timing and targeting fungicides to the ear during the critical infection period at flowering time. Another challenge is that the timing of fungicide application differentially affects FHB disease and even mycotoxin content of the grains [69]. Furthermore, during the past decades, fungicide resistance became more and more a central concern, and different fungicides or mixtures of fungicides of different classes were used and tested against FHB (Table 6).

Table 6: Fungicides tested against FHB

Fungicide class	Fungicides
MBC	thiabendazole
DMI	metconazole, prothioconazole (proline), tebuconazole
QOI	pyraclostrobin, trifloxystrobin, azoxystrobin
SDHI	isopyrazam, benzovindiflupyr (solatenol), fluxapyroxad, boscalid, pydiflumetofen, bixafen, fluopyram, penflufen, penthiopyrad, sedaxane

Greatest potential for success was shown with demethylation inhibitors (DMIs) and DMI co-formulations [28, 70-72]. Metconazole, prothioconazole, tebuconazole and their mixtures with pyraclostrobin and trifloxystrobin can be used in FHB control. In 2004, prothioconazole was introduced and this triazol fungicide had the best inhibitory activity against *F. graminearum* in field experiments [73].

As a consequence of the wide use of these available fungicides, resistance has emerged and needs to be considered. Natural tolerance of *F. graminearum* to QoIs [74] and resistance to MBC fungicides [75] were shown, which limit the use of these fungicides against *Fusarium* species. Experiments were done to identify the ability of prothioconazole (Proline, Bayer Crop Science) at three timings to reduce FHB and DON production [73]. Results showed that prothioconazole controlled FHB development and reduced DON production in field even at application timing before flowering. In contrast, other studies showed that prothioconazole timely induced hydrogen peroxide production by *Fusarium* which increased DON accumulation [76]. Most of the current SDHIs, do not have an activity towards FHB, especially against most of *Fusarium* sp.

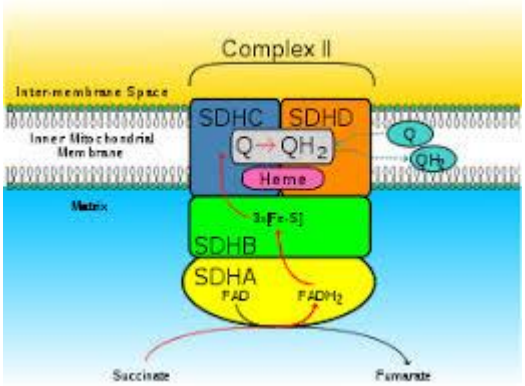

Figure 12: Succinate dehydrogenase enzyme and its three subunits B, C and D (Wikipedia)

1.4.2. Succinate dehydrogenase inhibitors

Resistance to SDHI has been of high interest as this family of fungicide is the youngest. The target site of SDHI is located in the mitochondrial respiratory chain of the fungus, named the complex II. Complex II has four subunits, but active ingredients of the SDHI-type only interact with the subunits B, C and D (Fig. 12). UV mutation conferring resistance to fluxapyroxad in *Z. tritici* were been identified in all three subunits [77] and seven mutations were associated with resistance [78]. Resistance to boscalid in *Botrytis cinerea* by site-directed mutagenesis on *SDHB* was found [79] and in total 21 mutations

(natural or lab mutants) were discovered [80]. Moreover cross-resistance relationships between SDHIs with similar chemical structures exist [81]. Despite the fact that *Fusarium* tolerance was shown towards isopyrazam [82], it seems that some mutations are associated to higher resistance factors [83].

Syngenta developed a new fungicide against FHB using the succinate dehydrogenase inhibitor (SDHI) mode of action. This new fungicide was named Adepidyn (APN; pydiflumetofen) and is able to bind into the pocket were other SDHIs are not binding and exerts an activity towards *Fusarium*.

1.4.3. Adepidyn™-Pydiflumetofen

Adepidyn™, with the active ingredient pydiflumetofen, is a novel broad-spectrum fungicide from Syngenta that offers effective and long-lasting disease control across multiple crops. Adepidyn™ delivers robust protection from a broad range of diseases in cereals. Performance has been excellent against Fusarium head blight and in the control of leaf spots including Septoria. It also provides excellent control across a range of important fungal diseases in corn, soybean, peanuts, vegetables, potatoes, grapes and fruit crops, protecting against leaf spots, powdery mildew, Botrytis and Sclerotinia [84]**.** This fungicide will be a key element in this study.

1.5. FHB in corn

FHB is also an issue in corn cultivation and causes severe disease on the ears (Fig. 13), together with yield loss. The entry of *Fusarium* sp. into corn ears occurs through wounds produced by insects or birds or by the growth of mycelium down the silks to the kernels, and infection is done by macroconidia or ascospores [85, 86]. Most common species which infect corn are *F. graminearum*, *F. proliferatum*, *F. verticillioides*, *F. subglutinans* and *F. cerealis* [25]. Mycotoxins which result from these infections are DON, zearalenone and fumonisin. Apart from finding new corn hybrids resistant to *Fusarium* and using insecticides to control FHB in corn, there is so far no effective control agent available. Also, there is no fungicide that is established as a regular and potent control tool.

As for wheat, corn development is described in a suite of stages (Fig. 14), with stages during the vegetative phase being abbreviated with V, and stages during reproduction being abbreviated with R. This stage scale is also used by farmers [87], and it will be used in this study to describe the development stages of corn.

Corn came from Mexico about 10 000 years ago and was introduced to Europe in 1494 by Christopher Columbus [88]. The species is in the family of the *Poacea*. Furthermore, it has a C4 photosynthesis system (assimilation of CO_2). Corn is one of three most commonly cultivated species in the world, and according to the FAO, it was the first grain producing 1017 million tons in 2013.

Besides susceptibility to *Fusarium*, corn is also susceptible to several other diseases such as Rhizoctonia, stalk rot as well as insect attacks [89].

Figure 13: F. graminearum *(left) and* F. verticillioides *(right) on corn ears (J. Mark)*

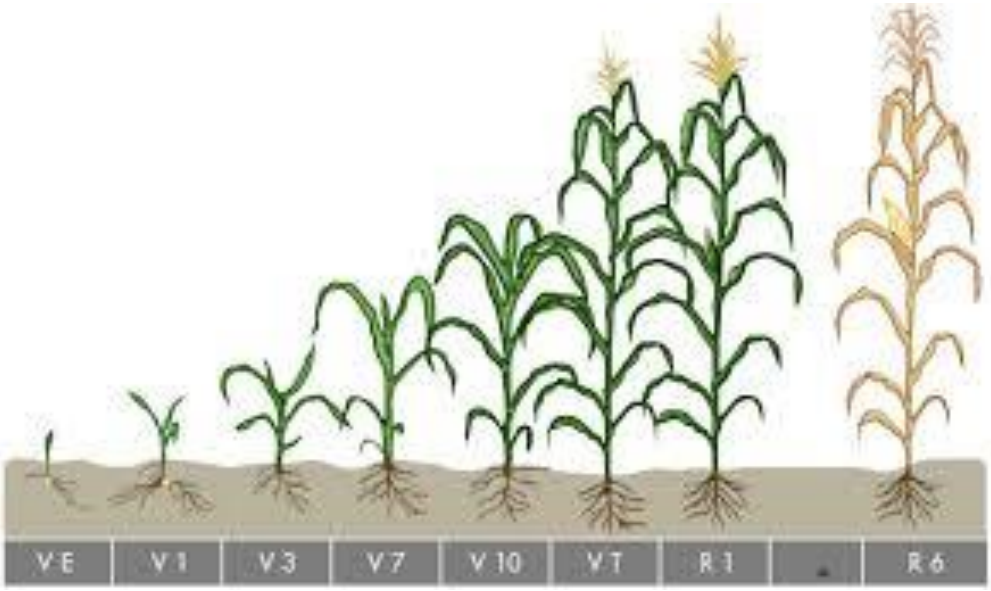

Figure 14: Growing stages of corn (Pioneer)

1.6. Aim of the work

The goal of the presented thesis was to study the interaction of fungicide action and FHB disease complex in order to provide insights helping the control of the FHB complex and its mycotoxins in wheat and corn. This thesis consists of four research chapters.

Chapter 2 aimed to characterize the FHB complex assigning to each isolate its species identity. A second goal was to investigate mycotoxin production and sensitivity to fungicides of the SDHI, DMI, QoI and MBC families on the level of populations within species. To achieve this ITS sequencing, mycotoxin analysis, and *in vitro* assays for the fungicide sensitivity were performed using a *Fusarium* panel including more than 500 isolates. This work allowed the later selection of interesting isolates and species in further studies, apart from getting a deeper knowledge of the panel.

Chapter 3 focuses on understanding differences in SDHI sensitivity among species. More specifically, was tested the hypothesis that *SDH* genes were responsible for differences in sensitivity among species. A second hypothesis was that sequence divergence in *SDH* subunits could provide a robust phylogeny. Molecular analysis, *in vitro* dose response assays and *in planta* assays were performed.

Chapter 4 describes a study on determining the optimal infection timing of *Fusarium* but also the optimal application timing of the SDHI-fungicide Adepidyn and how this might influence the FHB complex. Long curative and long preventive assays *in planta* were performed using greenhouse conditions. After harvest of the ears, analyses of symptoms and mycotoxin production were done.

The goal of Chapter 5 was to assess the efficacy of the fungicide Adepidyn under field conditions. The study was performed on wheat and corn and included preventive and curative treatments. Plants were inoculated in the field with *Fusarium* using different methods and mycotoxin amounts were analyzed.

2. Fungicide sensitivity of *Fusarium* isolates

2.1. Introduction

Pathogenic attacks by microorganisms is one of the most limiting factors in agricultural crop production [90]. Pathogenic infection is based on the biotic interaction between plants and microorganisms, but further determinants of infection typically include abiotic factors. Infection of plants by fungi and bacteria are highly dependent on the environment and the climate [30, 91]. Europe for example is very favorable to fungal infection in agroecosystems due to wet environments and medium temperatures, especially during crop growing periods. Nevertheless, crop production is high in Europe, with e.g., 300 million tons of production of wheat, corn and barley [5]. It is a must to keep that production level as the demand is increasing. But high production level can only be achieved by efficient plant protection against pathogens and the diseases they cause.

On cereals, diseases can be multiple and one important is *Fusarium* head blight disease (FHB) complex. FHB is a severe disease on cereals and is a complex of several species infecting corn, wheat and barley. Among them the most abundant are *Fusarium graminearum, F. poae, F. tricinctum, F. culmorum, F. avenaceum, F. equiseti, F. sporotrichioides, F. langsethiae, F. verticillioides, F. proliferatum, F. subglutinans, F. cerealis* and *F. acuminatum [18, 19]*. The disease is of economic importance, as its development on ears causes a loss of grain yield and quality, and in very bad cases, it can lead to complete destruction of the harvest. The major problem of *Fusarium* is not only that it causes visual lesions, but also its production of mycotoxins that are highly toxigenic for humans and animals [24]. The control of this disease is difficult because of the different species associated to FHB and the mostly unique mycotoxin panel produced by each species. Mycotoxins include deoxynivalenol (DON), nivalenol (NIV), HT-2 and T-2 zearalenone (ZEN) and fumonisins.

Options of controlling *Fusarium* include good agricultural practice such as crop rotation, tilling and growing resistant cultivars [71]. Using all these practices help to secure yield potential, but the predominance of monoculture still requires the application of fungicides to save the harvest. Securing yield quantity and quality is important for the farmers, and this is why fungicide development is of great interest and using chemical plant protection seems the easiest way to achieve efficient and consistent control over potential disease. The desire of consistent control is especially important given that the severity of disease depends typically on the year and the climatic conditions [30]. Using fungicides is a save way to control pathogens as each culture faces variability in general climate and particular weather events. Demethylation inhibitor (DMI) fungicides and quinone inhibitor (QoI) fungicides were reported to decrease FHB sp. *Fusarium* [72]. However, decreased fungicide sensitivity

or full fungicide resistance has been reported to these two fungicide classes [68]. Therefore, the new class of SDHI (succinate dehydrogenase inhibitors) fungicides has gained attractiveness, particularly those with novel chemical structures [80].

The aim of this work was to describe a representative panel of *Fusarium* isolates of FHB and assess the sensitivity of them in *in vitro* assays to different fungicides, including pydiflumetofen, a new chemical substance falling in the class of SDHI fungicides. Furthermore, mycotoxins of different species were studied to increase the knowledge of the panel for further assays. The panel included over 500 *Fusarium* isolates isolated from wheat, barley and corn, and included 13 species. Each isolate was characterized by ITS (internal transcribed spacer) sequencing for species identification.

2.2. Material and methods

2.2.1. Origin of material

In total 539 *Fusarium* isolates were used for the study and named CS-FU00001 to CS-FU00539 (Table 1 in Appendix). Each isolate was isolated either from wheat, barley or corn kernels, and two samples of each isolate were put at -80°C to have a stockpile. Seven isolates did not grow on the Agar plate at reception. Finally, assays were performed on 532 isolates.

2.2.2. Species identification using ITS sequencing

2.2.2.1. DNA Extraction

Fungal isolates were grown on plates with PDA medium (potato dextrose agar, 39 g/L) for 12 days at 65% relative humidity (HR), 12 hours dark and 12 hours UV light, 21°C. Then mycelium was taken from the plates with a clamp and put in a collection microtube. To avoid cross-contamination, the mycelium was put on the bottom of the tube. Microtubes were covered with parafilm and samples were lyophilized for 6 hours. A tungsten carbide bead and 300 µl of Buffer RLT (MagAttract 96 DNA Kit, Hilden, Germany) were added to each collection microtube. Tubes were sealed with the caps. The carbide had to be able to move in the tube. Samples were shaken two times for 1 minute at 30 Hz in TissueLyser (Qiagen, Hilden, Germany). Finally, the tubes were centrifuged for 5 minutes at 6000 x g. The DNA extraction was performed following the protocol of Magattract Kit (Magattract HMW DNA Kit, Qiagen). Samples were stored at -20°C if not used. In total, 532 DNA extractions were done. Quality of the DNA, assessed by the ratio of absorption at 260/230 [nm], ranged from 1.96 to 2.12.

2.2.2.2. PCR performing

Isolates were identified using ITS (internal transcribed spacer) sequencing, a rapid and efficient method for identifying more than 500 isolates. Reference sequences (Table 1) used for this work had been established by Florian Walder and collaborators [40]. Primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and LR6 (CGCCAGTTCTGCTTACC) [40] were used for PCR reactions in a total volume of 50 µl. Containing 1 µl genomic DNA (<0.5 µg/50 µL), 35.75 µl dd water, 10 µl 5x GoTaq G2 buffer, 1 µl dNTPs 10 mM, 1µl primer ITS1F 10 mM, 1µl primer LR6 10 mM, and 0.25 µl Go Taq G2 Polymerase 5 U/ µl (provided by Promega Corporation). Primers were provided by Microsynth (Balgach, Switerland). The amplification reactions were done on a Thermocycler Bio-Rad (California, United States), using following steps (Table 2).

Table 1: Reference species used in the study for ITS identification

Species	Reference sequence
F. avenaceum	F. avenaceum(0380) [40]
	F. avenaceum(0379) [40]
F. cerealis	F. crookwellense(11081) [40]
	F. crookwellense(8125) [40]
F. culmorum	F. culmorum(9712) [40]
F. equiseti	F. equiseti(10015) [40]
	F. equiseti(05005) [40]
	F. equiseti(11034) [40]
F. graminearum	F. graminearum(0410) [40]
F. langsethiae	F. langsethiae(0420) [40]
F. oxysporum	F.oxysporum(07040) [40]
F. poae	F. poae(07027) [40]
	F. poae(0338) [40]
	F. poae(0378) [40]
F. proliferatum	F. proliferatum(05010) [40]
F. sporotrichioides	F. sporotrichioides(7044) [40]
F. subglutinans	F. subglutinans(7043) [40]
	F. subglutinans(07038) [40]
F. tricinctum	F. tricinctum (07015) [40]
	F. tricinctum (05009) [40]
F. venenatum	F.venenatum(11020) [40]
F. verticillioides	F. verticillioides(05007) [40]

The PCR products were separated on a 2% agarose gel diluted into TAE x1 buffer, adding 10 µl/ 100 ml GelRed dye. Electrophoresis (system Biorad) was performed at 140 V for 45 min. The length of the product was about 1.6 kb.

Table 2: PCR steps

Step	Temperature	Time
Initial denaturation	95°C	2 min
Denaturation	95°C	30 sec
Annealing	51°C	30 sec
Extension	72°C	30 sec
Final elongation	72°C	5 min

2.2.2.3. Sequencing of ITS region

The sequencing of each product was done by Microsynth (Balgach, Switzerland). The consensus of the sequences and analysis were done using Lasergene 14 software and the alignment of the sequences was done using MegAlign software (Madison, USA). Settings of the alignments included a clustal W alignment and were compared to the alignment done by Walder *et al*. (reference species were sequenced by Sanger method by Microsynth) [40]. The relationship of the different reference species, using MegAlign software, are shown in the Appendix Fig. 1, and the percentage of identity between each species is in the Appendix Fig. 2.

2.2.3. *In vitro* assays

Fusarium isolates were grown on PDA plates for 12 days at 65% HR, 12 hours dark and 12 hours UV light, 21°C.

In a round-bottom 96 well plate (design of the plate in Fig. 1), a stock solution of 5.5 µl a.i diluted in DMSO (dimethylsulfoxid) was prepared. The final concentrations in the plate ranged from 0.00011 to 20 ppm in 12 dilution step rates in lanes B to G, and the dilution steps were of factor 0.33. Lanes A and H were free of fungicide and used for check (medium+ pathogen only).

Figure 1: Design of the plate used for the in vitro *assay*

The dilution steps (Fig. 2) of wells B1, C1, D1, E1, F1, and G1 of the stock solution plate (each lane was another fungicide; six fungicides per plate were tested) were done with a Tecan robot (INSTRUMENT FREEDOM EVO 100 MCA96, S/N: 912000064, 2010, Innsbruck, Germany) and driven by the software Evoware 2.5 SP1 standard, program NodataMPS96pipettmulti. A first dilution was done in the stock solution plate by adding a volume of 269.5 µl 0.025% Tween 20 water in each well (dilution factor 50 to get a concentration of 200 ppm). After mixing, the robot transferred 10 µl of fungicide solution into a new plate called the assay plate. The a.i. (active ingredient) concentration ranged from 200 to 0.0011 ppm (10 fold higher than the final concentration). Two plates were prepared for each isolate and typically up to 60 isolates were tested in parallel (120 assay plates per run). Fungicides used for the *in vitro* assays are listed in Table 3.

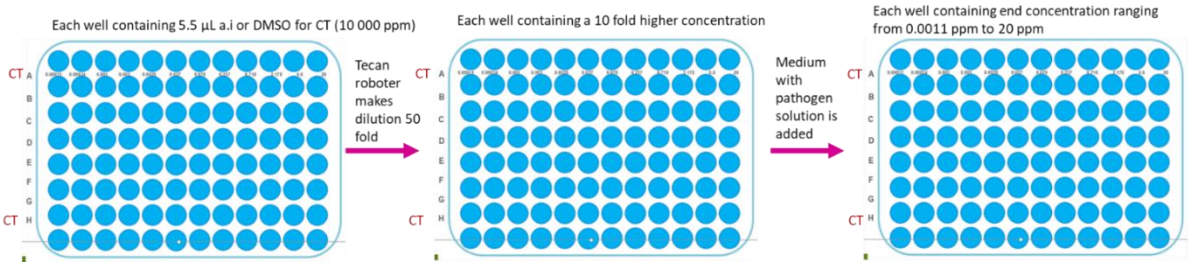

Figure 2: Fungicide dilution steps for the in vitro *assay*

Table 3: Fungicides used for the sensitivity assay

Fungicide	Mode of action	Molecular weight (g/mol)	Substance registration date
Pydiflumetofen	SDHI	426.68	2006
Benzovindiflupyr	SDHI	398.24	2012
Prothioconazole-desthio	DMI	312.2	2006
Tebuconazole	DMI	307.83	1986
Pyraclostrobin	QoI	403.39	2002
Azoxystrobin	QoI	387.82	1997
Thiabendazole	MBC	201.25	1971

For 120 assay plates, 2 L of AE media were needed (20 g yeast extract, 1g MGSO4*7 H_2O, 12 g $NaNO_3$, 1 g KCL, 3 g $KHPO_4$, 40g bacto agar, 40 mL glycerol). The solution was autoclaved for 20 minutes at 121°C. 90 µl of media was pipetted with a 12 canal multipipett into each well of each plate containing the 10-fold higher fungicide. Sterile bottles were filled with 5 ml sterile water. A 200 µl pipett was used to rack about 0.5 cm^2 of the pathogen plate by aspiring up and down several times 200 µl of medium and put into the bottle. The spore concentration had to be checked as we wanted it to be between 10 000 and 30 000 spores/ml. If the concentration was too low, the step was repeated by aspiring several times and racking the plate. If the spore suspension was too high, the spore suspension was diluted.10 µl of the spore suspension into each well of each assay plate was added. The OD was measured at t0 of each well at 620 nm and the assay plate was put at 24 °C dark, 90% HR for 72 hours.

After three days, OD at t72 OD was measured at 620 nm in each well. A macro in Excel was used to calculate the t72-t0 OD values for each well for each assay plate. These values were used to calculate the half maximal effective concentration, EC_{50}, for each fungicide using GraphPad Prism software (v8.2.1, established by Dr. Harvey Motulsky). The data was transformed to a semi-logarithmic formula of X= log (concentration a.i) and Y = OD t72- OD t0. For curve fitting, the Hillslope was set to -1.5 and the minimum and maximum of the asymptote were not defined. The assays were typically performed twice at a different time and with independent spore suspensions from different PDA plates. Data points considered as outliers by outlier visual detection were excluded from analysis; of 27 664 data points, 2% were removed due to outlier status. For each isolate, we got four EC_{50} values, and a mean EC_{50} was calculated.

Statistics were performed using RStudio software by performing pairwise Wilcoxon rank sum tests (2011, Boston, USA). Testing between the fungicides levels was done, and significance of correlations was adjusted for multiple testing. ANOVA could not be performed because variance of the EC_{50} values could not be transformed to a Gaussian distribution. A mean EC_{50} value was calculated for

each isolate and for each fungicide and statistics were done ($p < 0.05$) to estimate if the trends observed between fungicides, species and fungicides within a same species were significant.

2.2.4. Toxinanalysis

Wheat grains were mixed with water in Erlenmeyers (100 g of kernels and 100 mL of water in each erlenmeyer) and each extract was autoclaved twice at 121°C. Wheat grains were infected *in vitro* with the specific isolate grown on PDA plate. Incubation timing was 14 days and the grains were dried under sterile conditions. Then the infected dried grains were sent to Qualtech group (Nancy, France) for toxin analysis. Species analyzed were *F. cerealis*, *F. culmorum*, *F. equiseti.*, *F. poae*, *F. sporotrichioides* and *F. verticillioides.*

2.3. Results

2.3.1. ITS identification of each isolate

After obtaining the ITS sequences of the 532 isolates, their previous identification based on other methods were confirmed or had to be revised. Of 532 isolates, 21% had not been identified correctly. The revised identification was performed based on sequence information of Walder *et al.* [40] for the different species. Two *F. venenatum* isolates were found but I excluded them for further assays as they were not representative enough in my panel. All 532 isolates could be identified (Fig. 3). although *F. tricinctum* and *F. avenaceum* could not be fully discriminated for all isolates (37%) and were blasted on NCBI for species assignment. Both species seemed highly phylogenetically related (1 nucleotide substitution per 100 residues (NS/100 R)). Interestingly, *F. tricinctum* was found to be separated into 3 clusters. Isolates of *F. verticillioides* were related within the species between 0.1 and 0.5 NS/100 R and were in an own cluster.

F. subglutinans isolates were also in an own cluster, and were related between 0.1 and 0.2 NS/100 R. The same scenario was found for *F. proliferatum* but this cluster had almost no substitution within the species. *F. verticillioides* and *F. subglutinans* were related to 1 NS/100R and this sub-group was related to 2 NS/100 R to *F. proliferatum*.

F. equiseti isolates were separated into three clusters. As described by F. Walder *et al*. two clusters including reference strains *equiseti* 10015 and *equiesti* 11034 (*equiseti*_1) or the second cluster including *equiseti* 05005 (*equiseti*_ 2). In this study it seemed that a third cluster is existing, which I named *equiesti*_3. They were all related to less than 0.1 NS/100 R. *F. equiseti* were related to 11 NS/100 R to *F. proliferatum*.

F. culmorum showed 2 clusters too, related to 0.1 NS/100 R, and they were related to *F. cerealis* (*F. crookwellensee*) by 0.2 NS/100R. Both were related to *F. graminearum* which had more variability in the phylogeny as isolates had more than 3 NS/ 100R within the species.

F. sporotrichioides isolates were related by 0.2 NS/100R to *F. langsethiae* and both were related to 12 NS/ 100 R to the group *F. graminearum-F. cerealis- F. culmorum. F. poae* was divided into two clusters and was related to 12 NS/ 100 R to the group *F. langsethiae- F. sporotrichioides*.

F. venenatum was closely related to *F. poae* with 1 NS/100R and *F. oxysporum* was more related to *F. subglutinans* (1NS/100R). Four isolates were not identified through this method, 57 NS/100 R and were blasted on NCBI (CS-FU00092, CS-FU0093, CS-FU00094 and CS-FU00269). The blast showed species relation to *F. tricinctum*.

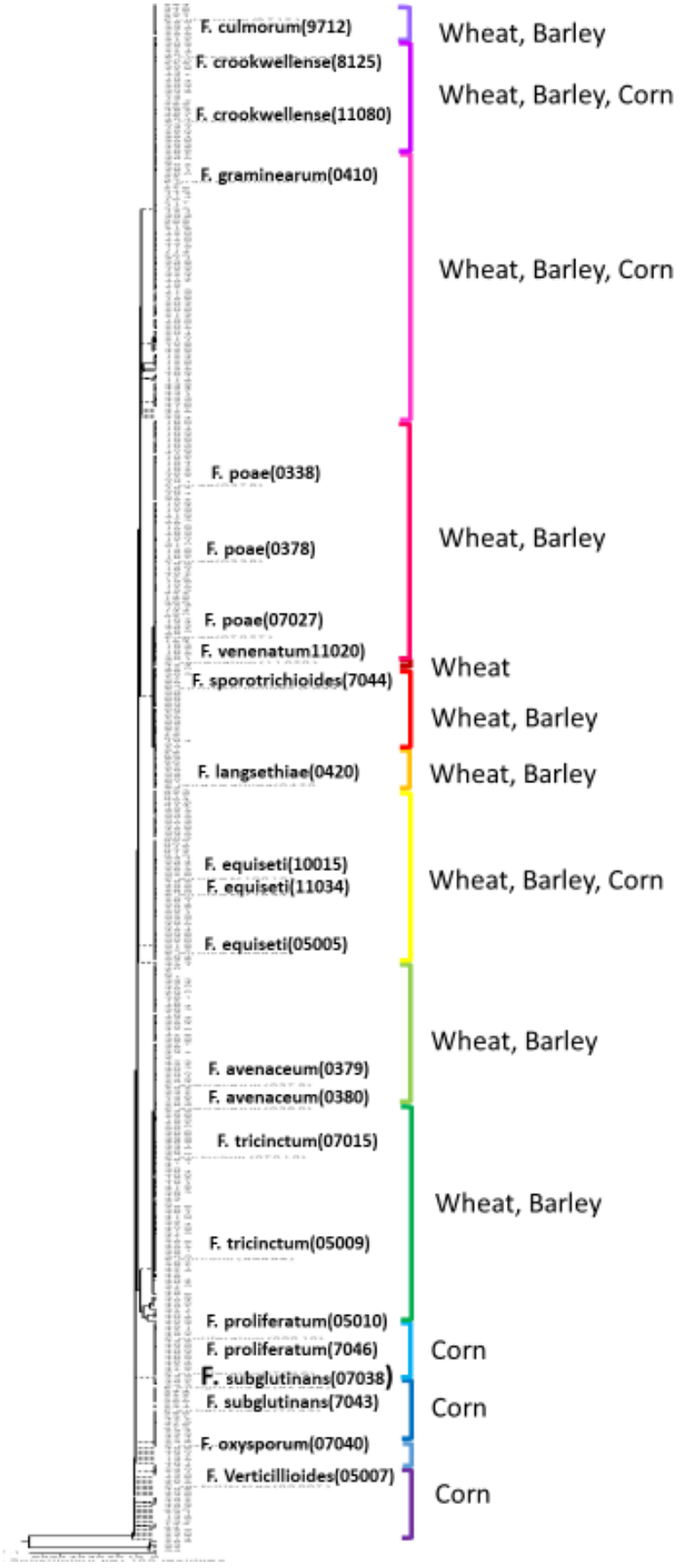

Figure 3: Phylogeny of 532 Fusarium *isolates based on* ITS and host crops of isolation

2.3.2. Panel description

The isolates came from 17 European countries and 13 species (Fig. 4; all information also in Appendix Table 1). Years of isolation ranged from 1964 to 2017. They had been collected on different hosts: barley 35.1%, corn 20.6%, wheat 26% and unknown 18.4%. Isolates came from different research institutions. They showed microscopically and macroscopically morphological differences. Spores had a ''croissant'' shape (Fig. 5A-B) and when grown on the plate, isolates differed in coloration ranging, from being pink, red, white, orange or brown (Fig. 5C). Some species also had a special smell when grown on agar plates, as does *F. poae* with a special peach smell. Growth and sporulation of each isolate were characterized by using three attributes: '' very good '', ''good'' and ''bad''. This information was needed for further greenhouse and field assays for which spore suspensions were used.

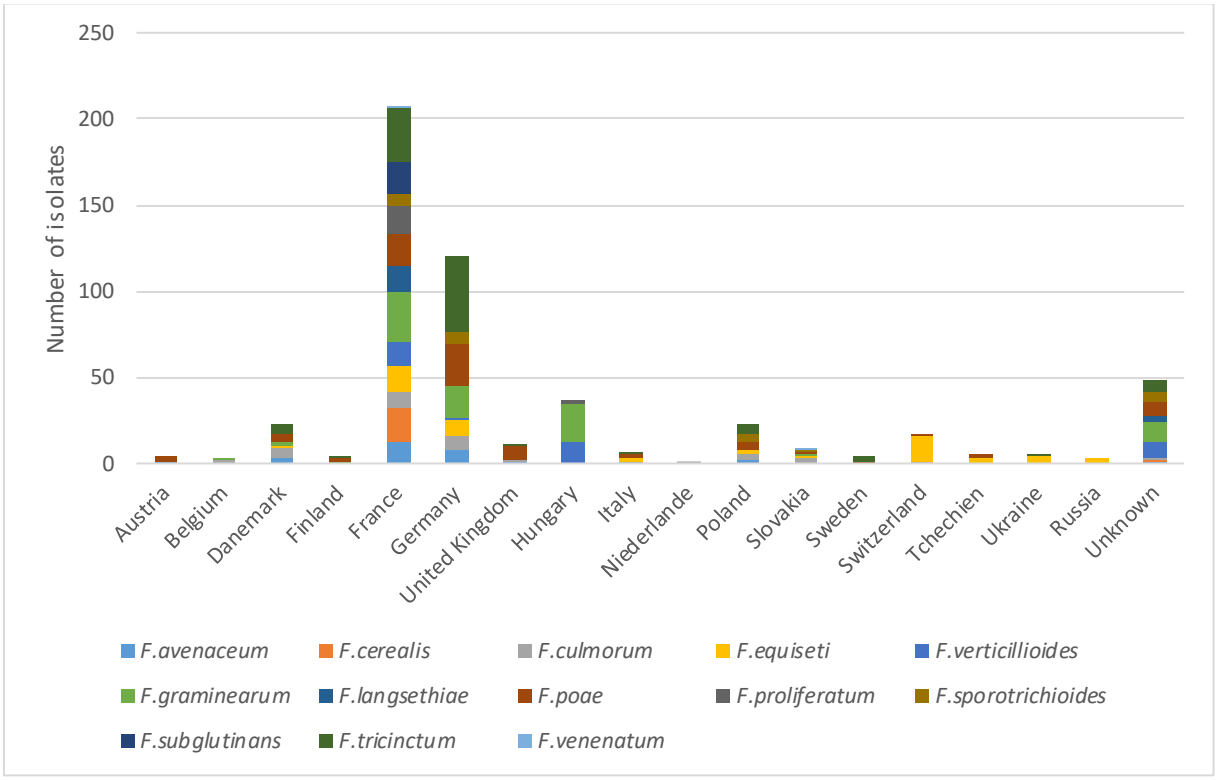

Figure 4: Number of isolates per species and countries

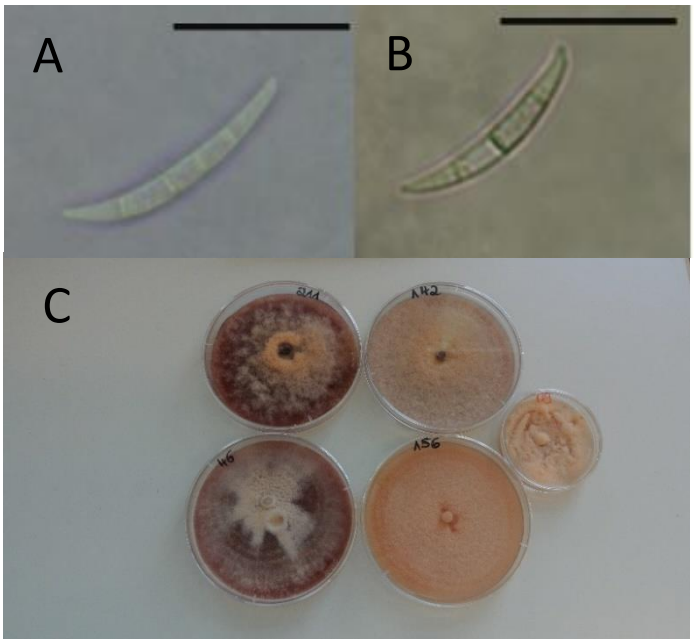

Figure 5: A. Macrospore of F. gram*inearum, scale bar 25 µm; B. Macrospore of* F. tricinctum, *scale bar 25µm; C. 211:* F. graminearum, *142:* F. verticillioides, *156:* F. poae, *46, 49:* F. tricicntum

2.3.3. Toxin analysis of different species

One hundred thirty-seven isolates were studied in depth by measuring which toxins they produce (Table 4). This step was important to have enough knowledge about each species and to select isolates for further assays. Some isolates were studied before having the ITS sequence and based on the species they were described. After ITS analysis, 11 isolates tested for mycotoxins were not the species meant. Results as measured showed that *F. langsethiae, F. graminearum, F. avenaceum, F. tricinctum* and *F. proliferatum. F. verticillioides* and *F. proliferatum* produced fumonisins. *F. cerealis* produced DON, NIV and fumonisin. *F. tricinctum* and *F. avenaceum* produced zearalenone, NIV and DON. *F. avenaceum* produced fumonisin only. *F. graminearum* produced DON, NIV, beauvericine, zearalenone, moniliformines but also T2/HT2. *F. sporotrichioides* was a T2/HT2 and beauvericine producer but some isolates also produced DON (79% of tested isolates), NIV (63%) and fumonisin (76%). *F. poae* also had two chemotypes with T2/HT2 and/or DON producers. The same was found for *F. culmorum*, which had two chemotypes. *F. equiseti* was a DON and NIV producer. Potential discrepancies to literature are discussed.

Species	NB of isolates tested	HT2toxine	T2Toxine	Somme T2+HT2	(3+15)-Acetyldeoxynivalenol	Deoxynivalenol	Nivalenol	FusarenoneX/FusarenoneX	Beauvericine/Beauvericin	Fumonisin B1	Fumonisin B2	B1 + B2	Fumonisin B3	Zearalenol	Moniliformin
F. cerealis	9														
F. culmorum	27	56%	56%	56%			89%	45%							
F. equiseti	21							19%							
F. langsethiae	4					50%	75%								
F. poae	63	29%	48%	48%	54%			62%							
F. sporotrichioides	19				58%	79%	63%	76%							
F. verticillioides	23														
F. graminearum	2														
F. avenaceum	2														
F. tricinctum	1														
F. proliferatum	2														

Table 4: Table 4: Mycotoxin production of some species. Red color show that the mycotoxin was absent in the analysis. Green color show that the mycotoxin was produced by the pathogen. Orange colors with the numbers show the percentage of the panel tested producing the mycotoxin.

2.3.4. *In vitro* sensitivity to fungicides

In vitro sensitivity to 7 active fungicide compounds were tested on 532 isolates covering of 13 species. Figure 6 shows the fungicide sensitivity of each species. *F. graminearum* was most sensitive (median 0.04 ppm) to pydiflumetofen, compared to other fungicides (median 0.06 to 3.35 ppm). Prothioconazole-desthio had the second-best efficacy (median 0.06 ppm) on the growth of *F. graminearum.* Thiabendazole had the lowest sensitivity (median 3.35 ppm), and sensitivities of pyraclostrobin/azoxystrobin and pyraclostrobin/benzovindiflupyr were in between and could not be differentiated statistically.

Sensitivities in *F. poae* were all significant except for azoxystrobin (not enough isolates tested for this species). Ranking from the highest to lowest overall sensitivity is as followed: pydiflumetofen (median 0.04 ppm) > prothioconazole-desthio (0.06) > benzovindiflupyr (0.14) > pyraclostrobin (0.7 ppm) > tebuconazole (1.11 ppm) > thiabendazole (2.50 ppm).

Sensitivities against *F. cerealis* showed no differences between prothioconazole-desthio/pydiflumetofen (median 0.05 ppm), tebuconazole/azoxystrobin (0.85 ppm and 0.13 ppm) and thiabendazole/azoxystrobin (2.41 ppm and 0.13 ppm). However, pydiflumetofen and prothioconazole-desthio had a better efficacy on the pathogen.

F. tricinctum was most sensitive against pydiflumetofen (median 0.03 ppm) followed by benzovindiflupyr (0.0.4) and then prothioconazole-desthio (0.12). Thiabendazole showed less efficacy (5.07 ppm) compared to all fungicides except for azoxystrobin.

Fungicide sensitivity of *F. avenaceum* differed among the active compounds, with a ranking from the most to the least efficient agent: pydiflumetofen (median 0.01 ppm), benzovindiflupyr (0.03),

prothioconazole-desthio (0.11 ppm), pyraclostrobin (1.07 ppm), tebuconazole (1.61 ppm), and thiabendazole (5.07 ppm).

Sensitivity of *F. culmorum* was different with another ranking: prothioconazole-desthio (0.04 ppm) > pydiflumetofen (0.07 ppm) > tebuconazole (0.50 ppm) > pyraclostrobin (2.69 ppm) > benzovindiflupyr (3.08 ppm) > thiabendazole (3.69 ppm).

Differences in sensitivity of *F. equiseti* were not significant between benzovindiflupyr/azoxystrobin, benzovindiflupyr /tebuconazole, pyraclostrobin/thiabendazole, benzovindiflupyr /thiabendazole and tebuconazole/thiabendazole. However, the pathogen showed again more sensitivity to pydiflumetofen than to prothioconazole-desthio (median 0.12 ppm to 0.15 ppm).

Sensitivity of *F. langsetiae* to thiabendazole could not be tested because of missing data, but there was a trend towards lowest efficacy. Moreover, differences between benzovindiflupyr/azoxystrobin, benzovindiflupyr/pyraclostrobin and tebuconazole/azoxystrobin could not be detected whereas prothioconazole-desthio showed better efficacy on *F. langsethiae* than pydiflumetofen (median 0.02 to 0.04 ppm).

Sensitivity of *F. proliferatum* to azoxystrobin could not be tested because of missing data, and sensitivities to benzovindiflupyr/pydiflumetofen, benzovindiflupyr/prothioconazole-desthio and tebuconazole/thiabendazole were not significant. But prothioconazole-desthio was better than pydiflumetofen (median 0.13 ppm to 0.19 ppm).

Differences in sensitivity of *F. subglutinans* to fungicides were all significant with a ranking starting from the best: prothioconazole-desthio (0.14 ppm) > pydiflumetofen (0.18 ppm) > benzovindiflupyr (0.3 ppm) > tebuconazole (0.9 ppm) > pyraclostrobin (2.55 ppm) > thiabendazole (4.81 ppm).

With only two isolates of *F. venenatum*, statistical analysis could not be performed.

Sensitivity of *F. verticillioides* to azoxystrobin could not be tested because of missing data and differences between tebuconazole/azoxystrobin and tebuconazole/ benzovindiflupyr were not significant.

Finally, sensitivity of *F. sporotrichioides* to pydiflumetofen and prothioconazole-desthio could not be differentiated, and thiabendazole had again the lowest efficacy against the pathogen.

Majority of isolates showed narrow sensitivity range, except for some highly sensitive isolates in species F. *graminearum*, F. *poae*, F. *tricinctum*, F. *culmorum*, F. *equiseti*.

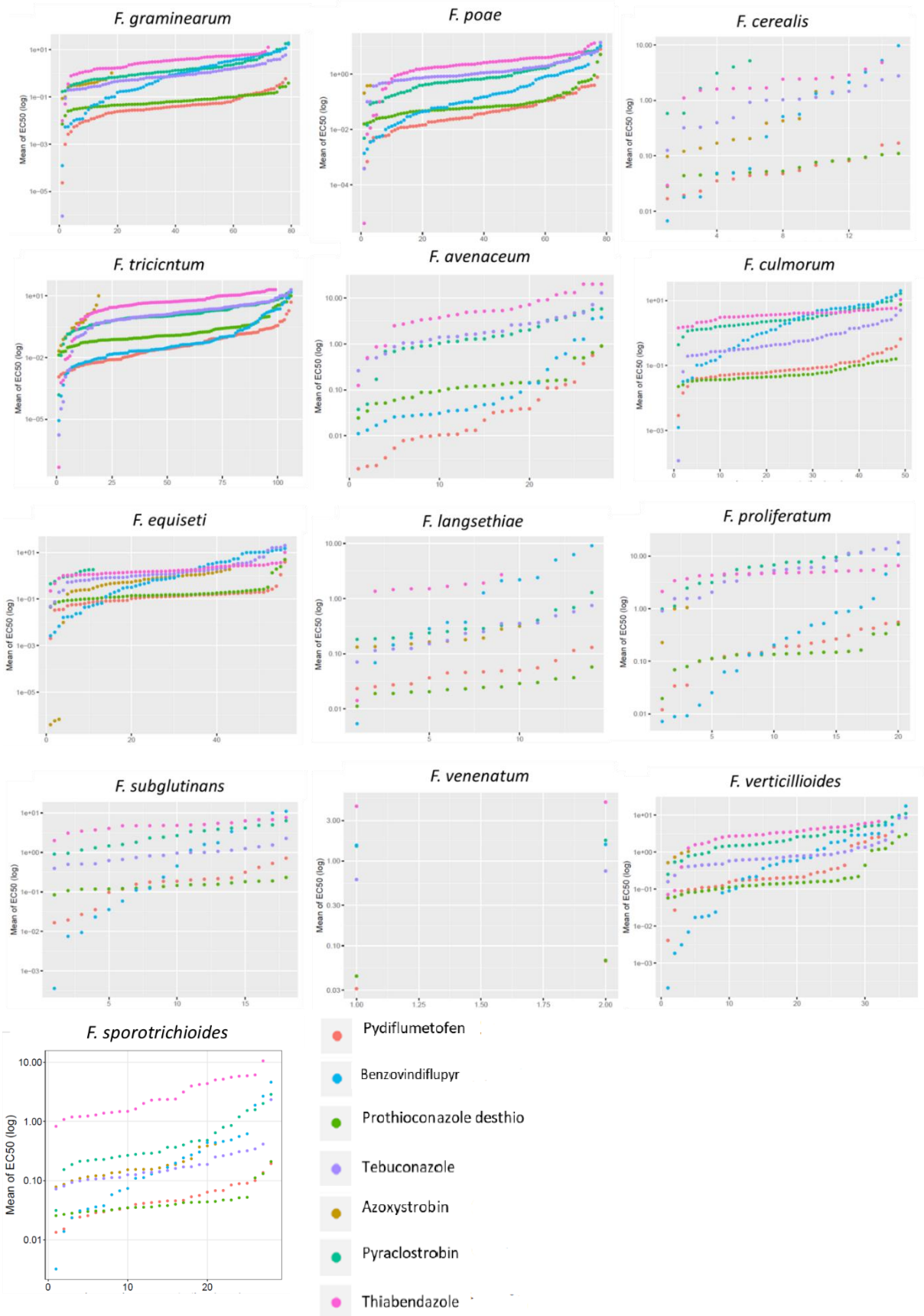

Figure 6: In vitro *mean EC_{50} sensitivities from lowest to highest values of* Fusarium *species against 7 fungicides. Abscises axis of each graph shows the number of isolates.*

As shown previously in Fig. 4, most species of the panel had been collected in France, Germany and Hungary. The distribution of the fungicides sensitivity at the species level in these countries was analyzed (Fig. 7). In France and Hungary, the differences between the fungicide sensitivities were the same and statistically different with following ranking from the most efficient to the least efficient: pydilfumetofen > prothioconazole-desthio > benzovindiflupyr > tebuconazole > pyraclostrobin > thiabendazole. In Germany sensitivities between prothioconazole-desthio and benzovindifluopyr could not be detected but pydiflumetofen had a better efficacy on *Fusarium* species compared to other fungicides. The distribution of all *Fusarium* species of all countries in regard to their fungicide sensitivities are shown in Fig. 8. Statistical analysis could not be performed as not all countries had enough species that represented them. The distribution showed that on a species level, there was some variation between the countries for fungicide sensitivity. Thiabendazole had the lowest variation among countries.

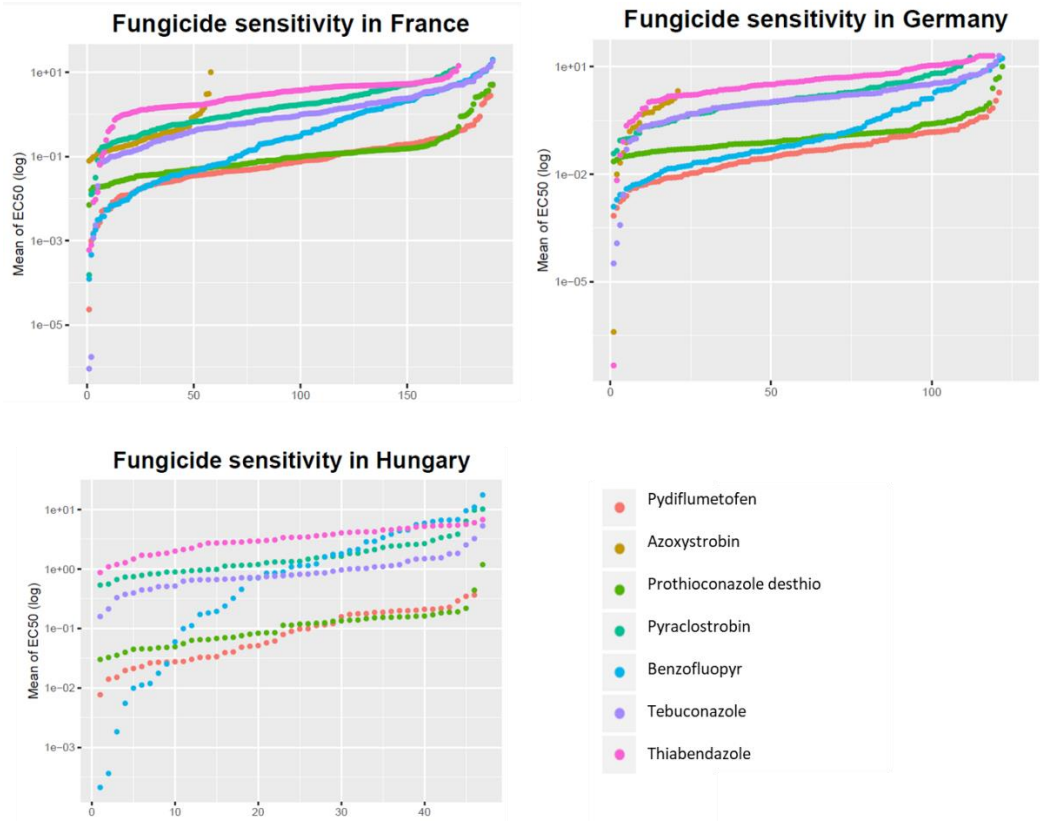

Figure 7: Fungicides sensitivity of Fusarium *species coming from Germany, France and Hungary. Abscises axis of each graph shows the number of isolates.*

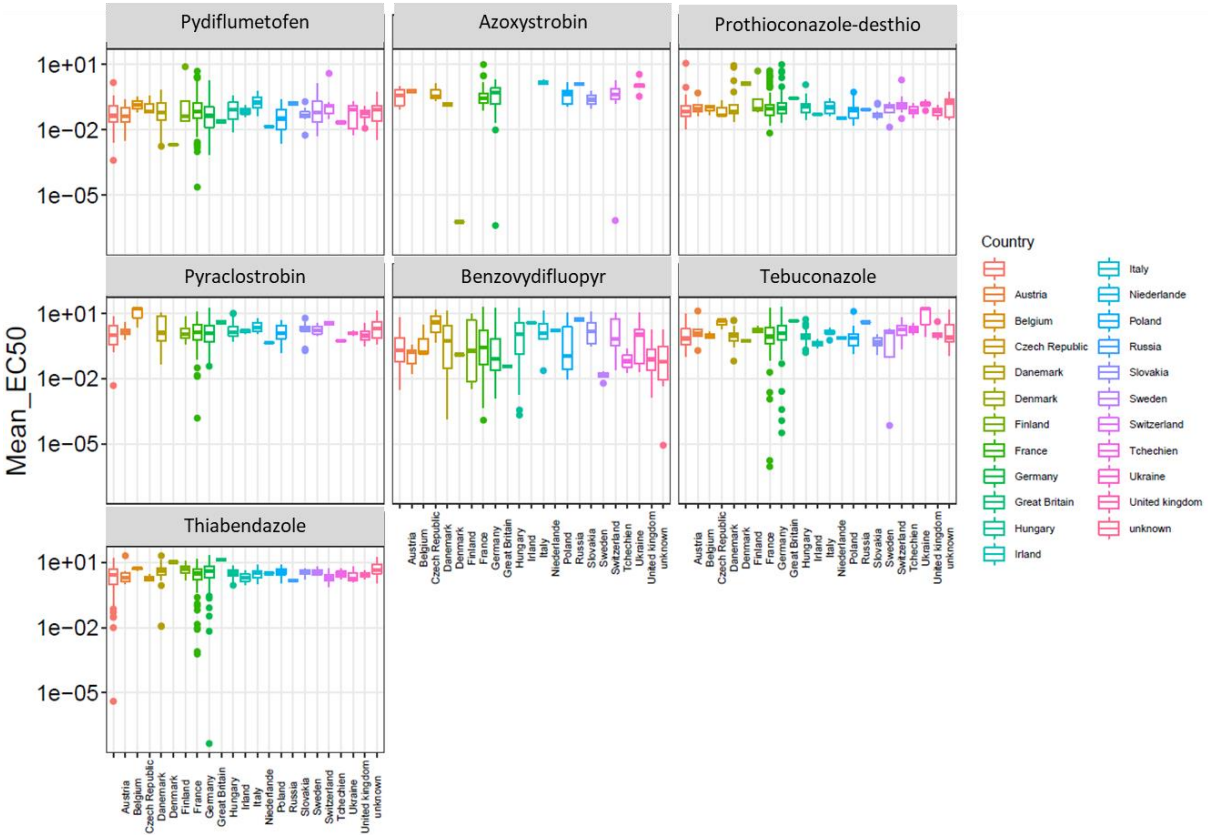

Figure 8: European representation of the fungicide sensitivity of Fusarium *species in each country*

2.4. Discussion

The first step of this study was to identify the *Fusarium* isolates of my panel. This was done by using ITS sequencing comparing my sequences with those of Walder *et al*. [40]. Twenty-one percent of the isolates of my panel had not been identified morphologically correctly at reception. They might have been identified by the owners by macroscopically characteristics, which is not as precise as molecular identification [35] . Other methods were used in the past as using specific primers of mycotoxin production genes to identify *F. verticillioides* from *F. proliferatum* [92], using RAPD for detection and identification of *F. culmorum* and *F. graminearum* [42]. Fungi in general have intron rich portions of protein coding genes [37] and several molecular methods are still developed for species identification among fungal pathogens such as *TEF-1α* [38] and *CYP51C* [39] amplification. However these methods are limited for certain *Fusarium* species [40] and as ITS analysis seemed to work globally it is an appropriate method for this study.

Walder and coworkers found two clusters of *F. equiseti*. In this study three were found and also sub-clusters for *F. tricinctum* and *F. culmorum* were found. Walder *et al*. did their study on 44 isolates and we did the ITS identification on a larger panel including 532 isolates. That means that the susceptibility of having more heterogenic isolates is higher and is more precise for the existing cluster identification. However, Walder's *et al*. reference genes should be enough for a unique species identification.

The diversity of mycotoxin production among *Fusarium* species is known and multiple studies have already be done to identify which mycotoxin is produced by which species [22, 24]. Although some species as *F. verticillioides* and *F. proliferatum* are known to produce always the same mycotoxins [25] it was already shown that species as *F. poae* are able to produce two or more mycotoxins depending on the isolate [23]. In the mycotoxin analysis we have done in this study it was also shown that *F. equiseti*, *F. culmorum*, *F. sporotrichioides* had in their population some isolate producing other chemotypes than their related isolates which is in correlation with other studies done [53] [93]. Having this knowledge it is of great interest not only trying to reduce *Fusarium* infections but also trying to control all of the mycotoxins which can be produced even if trichothecenes like DON are the most produced mycotoxins within cereals [41]. In this study I could not show if the differences within a species in mycotoxin production might be associated to the sub-clusters formed in the ITS phylogeny.

In vitro sensitivity studies allowed to get a global and precise overview on how the sensitivity to fungicides varies among *Fusarium* species. Exploiting results on species level, we were able to differentiate sensitivities within a same species. Thiabendazole showed the lowest efficacy against all *Fusarium* species followed by azoxystrobin, pyraclostrobin and tebuconazole. Pydiflumetofen was as

good as prothioconazole-desthio on *F. sporotrichioides* and *F. cerealis,* and clearly better on *F. graminearum*, *F. poae*, *F. avenaceum, F. tricinctum, F. equiseti* compared to other tested fungicides. Prothioconazole-desthio was better on *F. culmorum*, *F. langsethiae*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides*. Other SDHI showed a differential inhibition of the species within the FHB complex. The fraction of sensitive isolates was low for benzovindiflupyr (SDHI) while all isolates were sensitive for pydiflumetofen (SDHI). The fraction of isolates sensitive to Benzovindiflupyr varied among species with a particularly low frequency in *F. culmorum* and *F. graminearum* and a high frequency in *F. avenaceum* and *F. tricinctum*. Sensitivities between fungicides within European countries varied but this was in correlation with the variation observed within a species and between the species. Other studies showed inhibition of mycelial growth of *F. graminearum* isolates using DMI fungicides and a lower sensitivity using QoI fungicides [70] which is in correlation with the results shown here. Moreover, a recent study was done on *F. asiaticum* infecting cereals in China and it was shown that Pydiflumetofen provided a strong fungicidal potency and an inhibition of mycelial growth *in vitro*. Own assays on petri dishes confirmed the high inhibition potential of pydiflumetofen on *Fusarium* species compared to other fungicides (Appendix Fig. 5). Low sensitivity to thiabendazole could be explained by reported resistance whereas sensitivity might be driven by a high tolerance of F. species against these inhibitors. Differential sensitivity among the SDHI might be explained by gene sequence differences (chapter 3). Despite some reports of reduced sensitivity to DMI the tested isolates showed high sensitivity against prothioconazole-desthio.

This study was needed as first step for the whole work done following. Thanks to the sensitivity identity card done for each species, mycotoxin analysis and capabilities of growing and sporulation, isolates could be selected for greenhouse, field and further *in vitro* assays. The next step was to understand the differences in sensitivity of *Fusarium* isolates observed between SDHI fungicides. As prothioconazole-desthio is used so far to control FHB in the field [73], it is taken as reference in the studies.

3. Understanding genetic variability of the SDH subunit genes

3.1. Introduction

The control of Fusarium head blight (FHB) remains of strategic agricultural importance and is done with a combination of several measures. These include: (i) cultural control with crop rotation, appropriate use of fertilizers, and weed control [71]; (ii) biological control with bio-control agents including bacteria and fungi [28]; (iii) genetic control with host plant resistance, e.g. through breeding for accumulation of resistance QTLs (actually there are 52 QTL known conferring FHB resistance) [28]; and (iv) chemical control, which is currently the most effective single control tool of FHB [71].

Fungicides against FHB have been used since more than four decades with a broad range of studies assessing their efficacy [72]. Greatest potential for a successful control was found with demethylation inhibitors (DMIs) and their co-formulation with other fungicides such as QoIs [28, 70-72]. Metconazole, prothioconazole, tebuconazole and their mixtures with pyraclostrobin and trifloxystrobin were shown to decrease infection symptoms [72]. Use of prothioconazole (DMI) is currently the most effective active ingredient on the market against FHB controlling both the phenotype and the mxycotoxin contamination [73]. As DMIs are currently the only fungicide class available and use of DMIs have recently experienced more or less severe challenge from regulatory authorities, it is important to investigate new control options, including fungicides with a novel mode of action for *Fusarium*.

The succinate dehydrogenase inhibitors (SDHIs) are recently the fastest growing group of fungicides to a broad spectrum of fungal targets. [80]. SDHIs (the newest compounds are also known as pyrazole carboxamides, or carboxamides) target the SDH enzyme in the mitochondrial respiratory chain of the fungus. The enzyme complex is composed of 4 subunits called SDHA, SDHB, SDHC and SDHD, but SDHIs only interact with the subunits B, C and D to inhibit the electron transport chain necessary for respiration [79]. Those fungicides have been used since 1966 [80]. Several new molecules active against a broader spectrum of fungi have been developed over the last decade. The first generation of SDHI molecules showed to control a narrow spectrum of plant pathogens with mainly a seed-care use. This group has been enlarged with novel fungicides with a broader range of diseases [80]. Within this group (SDHI), the Fungicide Resistance Action Committee (FRAC) listed in 2019 a total of 23 different active ingredients from nine different chemical groups of SDHIs [83]. SDHIs are threatened by resistance evolution and eight of the nine chemical groups are already listed to be affected at least partially by resistance in fungal pathogens (FRAC) [80]. In general, all compounds with SDHI mode of action are cross resistant to each other. However, specific mutations could lead to

different resistance risks between the SDHIs and it was suggested that carboxin-selected resistant mutants might be controlled by structurally different SDHI compounds [94]. Mutants with decreased sensitivity to carboxamides fungicides induced under laboratory conditions or collected in the field have been described for various plant fungi [80]. UV mutagenesis in house was also done on *F. graminearum* to compare the impact of target mutations towards the different class of carboxamides [77]. SDHI fungicides tend to induce specific mutations in homologous locations in the SDH subunits in different fungal species. 27 amino acid substitutions on the 3 subunits B, C and D were identified in total among fungal pathogens and in most pathogens more than one mutation seem to be selected under field conditions but they rarely occur together [80]. These mutations indicated settle differences in the binding interaction of SDH subunits with specific fungicides. The latest SDHI fungicide included in the FRAC list is placed in a distinct chemical group group designated N-methoxy-(phenyl-ethyl)-pyrazole-carboxamides is pydiflumetofen (ISO name). Adepidyn™ (short form APN) is a protected brand name for the same compound. It was developed by the company Syngenta. Pydiflumetofen is a very broad-spectrum fungicide with an application in multiple crops, with a step change increase in activity for *Fusarium* species control as compared to other available SDHI fungicides.

The aim of this study, was to i) rationalize the different SDHI fungicide sensitivities of species found in the previous Chapter 2, by investigating the SDH subunit gene sequences, ii) test if there is a correlation for the SDHI sensitivity among a panel of 10 commercial SDHIs on selected isolates (isolates with low, mid and higher sensitivity to pydiflumetofen) iii) understand if difference of sensitivity to pydiflumetofen *in-vitro* do influence the *Fusarium* symptoms control *in planta*, iv) investigate the phylogeny of different species based on *SDH* genes. For that we attempted to sequence all three subunits genes of the 13 *Fusarium* species used in this thesis and compared the *in vitro* sensitivity to 10 SDHIs of 5 *Fusarium* species (6 isolates each): *F. graminearum*, *F. culmorum*, *F. equiseti*, *F. sporotrichioides* and *F. poae*. Some of these isolates were also used for dose-response assays *in planta* to understand differences in sensitivity at the plant level.

3.2. Material and Methods

3.2.1. *SDH* amplification and sequencing

Fungal isolates were grown on PDA (potato dextrose agar, 39 g/L) plates for 12 days at 65% HR, 12 hours dark and 12 hours UV light. After 12 days of growth mycelium was taken from the plates with a clamp and put it in a collection microtube. Great care was taken to put the mycelium at the bottom of the tube to avoid any cross contamination. Microtubes were covered with parafilm and samples were

lyophilized for 6 hours. A tungsten carbide bead was added to each collection microtube and 300 µl of Buffer RLT (MagAttract 96 DNA Kit, Qiagen) was added. Tubes were sealed with the caps. Samples were shacked two times for 1 minute at 30 Hz in TissueLyser (Qiagen, Hilden Germany). Finally, the tubes were centrifuged for 5 minutes at 6000xg. The DNA extraction was performed following the protocol of Magattract Kit (Magattract HMW DNA Kit Qiagen). Samples were stored at -20°C if not used. In total 532 DNA extractions were done Quality of the DNA, assessed by the ratio of absorption at 260/230 [nm], ranged from 1.96 to 2.12.

3.2.1.1. PCR performing

The three SDH-subunit genes, *SDHB*, *SDHC* and *SDHD*, were amplified using primers listed in Table 1) [82]. PCR reactions were done in a total volume of 50 µl. Containing 1 µl genomic DNA (<0.5 µg/50 µL), 35.75 µl dd water, 10 µl 5x GoTaq G2 Buffer, 1 µl dNTPs 10 mM, 1 µl primer ITS1F 10 mM, 1 µl primer LR6 10 mM and 0.25 µl Go Taq G2 Polymerase 5 U/ µl (provided by Promega Corporation, Madison, USA). Primers were provided by Microsynth (Balgach, Switzerland). The amplification reactions were done on a Thermocycler Bio-Rad. Using following steps Table 2.

Table 1: Primers used for SDH amplification

Gene	Forward primer [82]	Reverse primer [82]
SDHB	CGAAGTTTGACTGTCCTTCTCC	CGATCAAGAAAATAATATTGCCAAG
SDHC	CGATGCTCGCTCAACGTGTT	GCAACTTGTATCATCCACTGCG
SDHD	GCGACAACACCACAAGAATC	TGCCAATAATATGCTTCCTTCA

Table 2: PCR steps for SDH amplification

Step	Temperature			Time		
Subunits	SdhB	SdhC	SdhD	SdhB	SdhC	SdhD
Initial denaturation	95°C	95°C	95°C	3 min	3 min	3 min
Denaturation	95°C	95°C	95°C	30 sec	30 sec	30 sec
Annealing	57°C	58°C	55°C	30 sec	30 sec	20 sec
Extension	72°C	72°C	72°C	1'20 min	50 sec	50 sec
Final elongation	72°C	72°C	72°C	7 min	7 min	7 min
Soak	4 °C	4 °C	4 °C	∞	∞	∞

Products were separated on a 2% agarose gel diluted into TAE x1 buffer, adding 10 µl/ 100 ml GelRed dye. Electrophoresis (system Biorad) was performed at 140 V for 45 min. The length of the product were about 1000 bp for *SDHB*, 700 bp for *SDHC* and 700 bp for *SDHD.*

3.2.1.2. Sequencing of SDH genes

Sanger sequencing of each product was outsourced to Microsynth. The consensus of the sequences and analysis were done using Lasergene 14 software and sequences were compared to available sequences on NCBI to get the whole sequence (Fig. 3, Appendix), the sequence without exons and another without introns. The alignment of the sequences was done using the MegAlign software (Madison, USA). Settings of the alignments included a Clustal W model and phylogeny tree, Neighbor-Joining algorithm was done using the same program.

3.2.2. *In vitro* assay

The *in vitro* assay performed for the *Fusarium* monitoring in Chapter 2, allowed us to select 6 isolates from *F. graminearum, F. culmorum, F. poae, F. sporotrichioides* and *F. equiseti*. For each species, two isolates were selected with a low, two with a mid and two with a high pydiflumetofen EC_{50} value (0.0004-1.1 ppm). *Fusarium* pathogens were grown on PDA (potato dextrose agar, 3 9 g/L) plates for 12 days at 65% HR, 12 hours dark and 12 hours UV light. The analysis was performed as in Chapter 2.

Fungicides used for the *in vitro* assays are listed in Table 3.

Table 3: Fungicides used in the assay. For each fungicide the mode of action, its molecular weight, substance registration date and the company by which the fungicide was developed.

Fungicide	Mode of action	Molecular weight (g/mol)	Substance registration date*	Company
Pydiflumetofen	SDHI	426.68	pending (2016)	Syngenta
Benzovindiflupyr	SDHI	398.24	2012	Syngenta
Prothioconazole-desthio	DMI	312.2	2008	Bayer
Bixafen	SDHI	414.21	2013	Bayer
Boscalid	SDHI	343.21	2008	BASF
Fluopyram	SDHI	317.8	2014	Bayer
Fluxapyroxad	SDHI	381.3	2013	BASF
Isopyrazam	SDHI	359.42	2013 (2010)	Syngenta
Penflufen	SDHI	317.41	2014	Bayer
Penthiopyrad	SDHI	359.41	2014	Mitsui
Sedaxane	SDHI	331.36	2014 (2010)	Syngenta

*Europe (and other countries). Source: EU Pesticides Database (and Syngenta)

3.2.3. Dose response *in planta*

Isolates used in this assay were the same as for the former *in vitro* assay (5 species x6 isolates). Fungal isolates were grown on PDA (potato dextrose agar, 39 g/L) plates for 12 days at 65% HR, 12 hours dark and 12 hours UV light, 21°C. After 12 days of growing a spore suspension of each isolate was prepared (Table 4). All dose response assays were performed using wheat plants. Wheat seeds, variety Monsun were seeded at 5 seeds per pot (mixed soil 2g of fertilizer per L of soil and (2-chloroethyl) trimethylammonium chloride (CCC) treatment 4 mL/L). After 2 weeks, seedlings were thinned to achieve 4 seedlings per pot. After 9- and 11-weeks plants were trimmed during growing to obtain only 4 main ears on 4 separate plants per pot. Plants were used for the assay at full flowering. Greenhouse conditions were 19°C night for 12 hours, 21°C day for 12 hours, 80% RH and plants were irrigated every day as needed. Each assay was performed twice at independent times. For each isolate 8 treatments were performed and 3 pots / 12 plants per treatments were used. Formulations used are EC62.5 for pydiflumetofen and EC250 (the product Proline) for Prothiocoanzole. Treatments are: check untreated and uninfected; check untreated infected; pydiflumetofen (=APN) 50 g/ha; APN 100 g/ha; APN 200 g/ha; proline = prothioconazole 50 g/ha; proline g/ha; proline g/ha.

All plants of one assay were treated at the same time using a Track Sprayer (Caromatic Swiss technology, 2006) and one day after treatment were inoculated with a spore suspension (Table 4). Plants were put in a climatic chamber for 2 to 4 days (Table 4) at 19°C, full dark and at 100% humidity.

Table 4: Spore suspensions for each species and incubation timing after inoculation

Species and isolates	Spore suspension concentration (sp/ml)	Days of incubation at 100% humidity
F. graminearum	200' 000	2
F. culmorum	200' 000	2
F. poae	400' 000	4
F. sporotrichioides	200' 000	2
F. equiseti	200' 000	2

After incubation, plants were put again in the greenhouse, irrigated every day and the disease was assessed after 10 and 14 days after the inoculation.

Data from the inoculated plants were subjected to analysis of variance using the Syngenta in-house package Ascapwin. The terms in the statistical model were treatment and block. Prior to analysis, the percentages were arcsin-transformed, i.e. $y=\arcsin\sqrt{(x/100)}$, so as to better meet the assumptions upon which the validity of the analysis depends. The statistical significance of the overall effect of treatment was assessed via an F-test. In cases where the F-test was significant at the

customary 5% probability level (i.e. F-test probability <5%), the significance of differences between specific treatments, including the inoculated check, was assessed using the LSD (Least Significant Difference) method. Means on the transformed scale that differed by more than the relevant LSD were significantly different at the customary 5% probability level, providing evidence of a genuine difference between the two treatments in question. Differences that were smaller than the relevant LSD were considered no greater than we would expect to see simply because of random variation, and therefore did not provide convincing evidence of a genuine difference between the two treatments in question. The outcome of all possible treatment comparisons is summarized in the form of a letter such that means with no letter in common indicate significant differences.

3.3. Results

3.3.1. Diversity of *SDH* sequences between species

Each sequence was aligned to the corresponding reference sequence to check integrity. The length of the different fragments are listed in Table 5. Not all subunits for all species could be amplified. *SDHB* was amplified and sequenced at least for species *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae*, *F. sporotrichioides*, *F. verticillioides*, *F. subglutinans*, *F. proliferatum*, *F. langsethiae* and *F. tricinctum*. *SDHC* sequences were finally available for *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae*, *F. sporotrichioides*, *F. verticillioides*, *F. subglutinans*, *F. avenaceum* and *F. tricinctum.* SDHD sequences were revealed for *F. poae*, *F. sporotrichioides, F. equiseti, F. cerealis*, *F. culmorum* and *F. graminearum.*

Table 5: Length of SDH *sequences*

	Sequence (bp)	**Coding sequence (bp)**	**Non-coding sequence (bp)**
SDHB	963	837	126
SDHC	674	564	110
SDHD	688	549	139

Alignments of the genomic sequences, non-coding sequences and coding sequences were performed (Fig. 1, 2, 3). For the *SDHB* subunit (Fig.1), phylogenetic analysis showed that the *SDHB* gene of *F. subglutinans*, *F. proliferatum* and *F. verticillioides* was related. *F. sporotrichioides* and *F. langsethiae* were in separated clusters but both were related to each other and both were related to *F. poae*. *F. graminearum* isolates formed an own cluster whereas *F. cerealis*, *F. culmorum* and *F. tricinctum* could not be differentiated from each other. *F. graminearum* and the *F. culmorum-F. cerealis-F. tricinctum* cluster, were more closely related to *F. sporotrichioides-F. langsethiae-F. poae*. Phylogeny of the genomic sequence and of the non- coding sequence showed similar relations, whereas the phylogeny of coding sequence resolved the species similarly well but was not able to properly identify relationship for F. *poae*, F. *langsethiae* and F. *sporotrhichioides*.

For the *SDHC* subunit (Fig 2) again all three types of sequence could resolve the species well. The genomic sequence gave the best result to represent the lineage of species, grouping *F. graminearum*, *F. culmorum* and *F. cerealis*, as well as *F. poae* with *F. sporotrichioides*, and *F. verticillioides* with *F. subglutinans*. These groups are expected from literature and ITS lineage. Amplification of *SDHC* for *F. tricinctum* and *F. equiseti* (not possible for *SDHB*) indicates that both species are more distant from the clusters named above, and also distinct from each other.

Interestingly, *SDHC* from one isolate of *F. avenaceum* could be amplified only. Also, this sequence grouped within *F. graminearum*. Based on ITS alignment and biology (toxins produced), the expectation for F. *avenaceum* should be to cluster with *F. tricinctum*. It remains therefore questionable, if this sequence may represent the amplification of a contamination, or if the original sample is a mixture of isolates containing both *F. avenaceum* and *F. graminearum* (no efforts were put into producing single spore isolates before DNA preparation).

Amplification *SDHD* was possible from the least number of species. Albeit able to resolve isolates at species level (Fig. 3), this subunit showed less variation in the phylogeny and less relationship between different species as compared to the other two subunits.

In each alignment there were about 5% +/- 2% of isolates which did not cluster within the correct species. It remains open if, as discussed above, this is the result of DNA being prepared from samples that do not represent pure isolates.

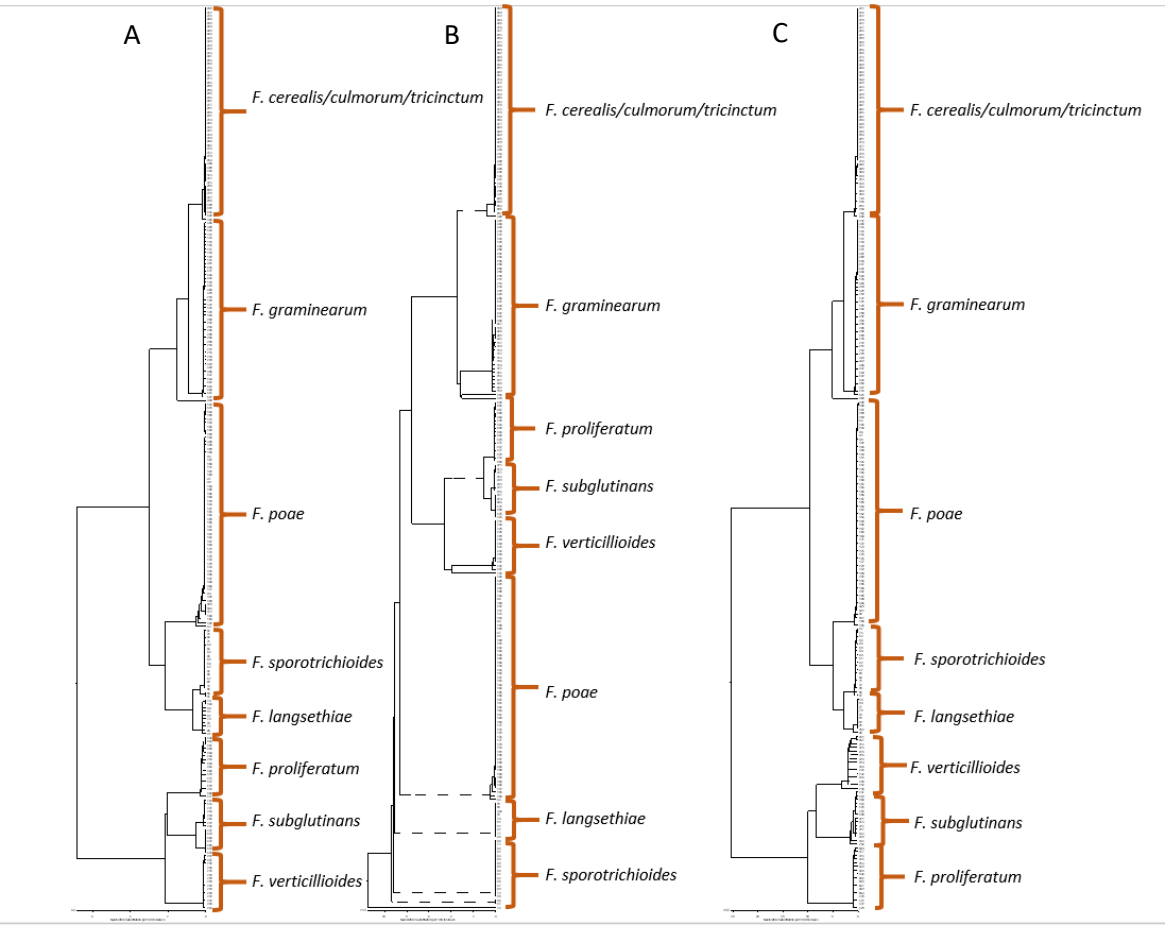

Figure 1: SDHB *trees; Neighbor-Joining algorithm. A: whole sequence, B: coding sequence, C: non-coding sequence*

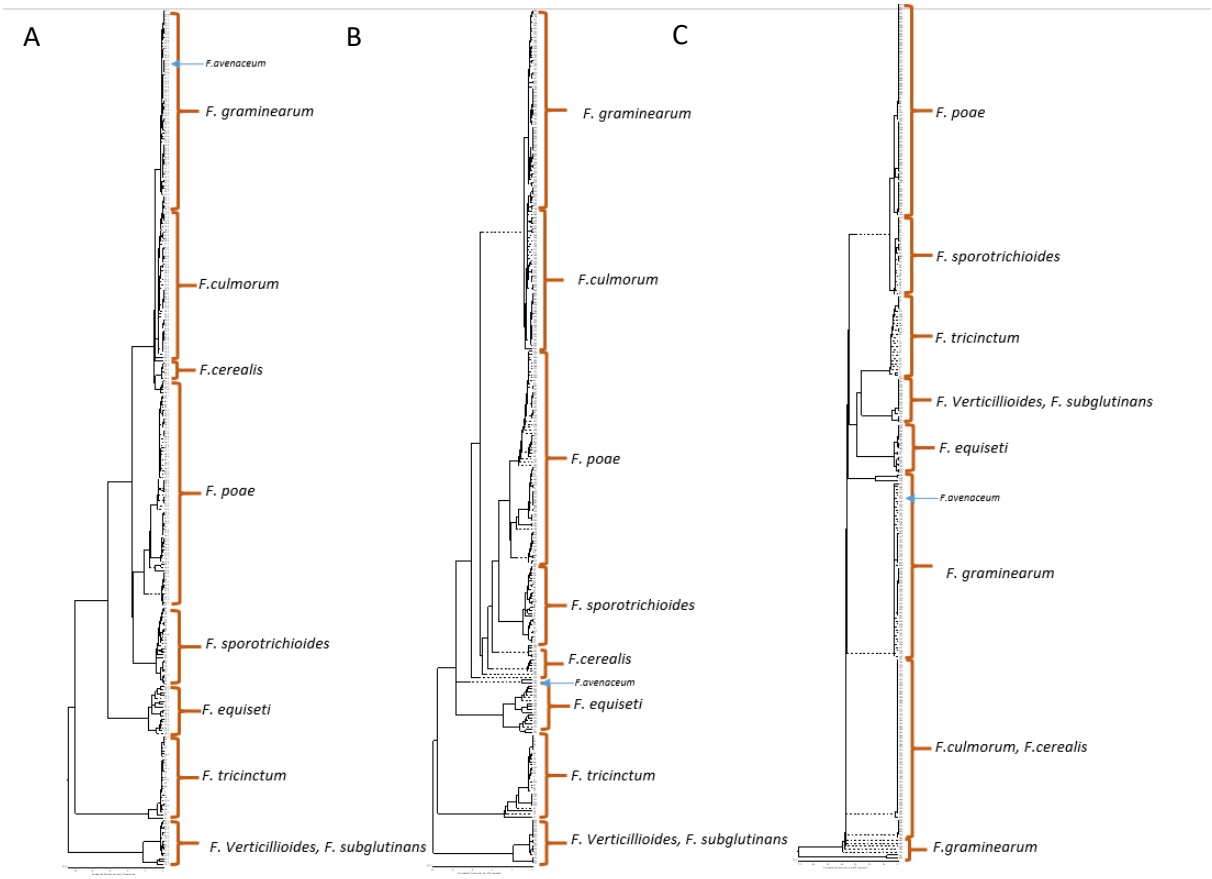

Figure 2: SDHC *trees Neighbor-Joining algorithm, A: whole sequence, B: coding sequence, C: non-coding sequence*

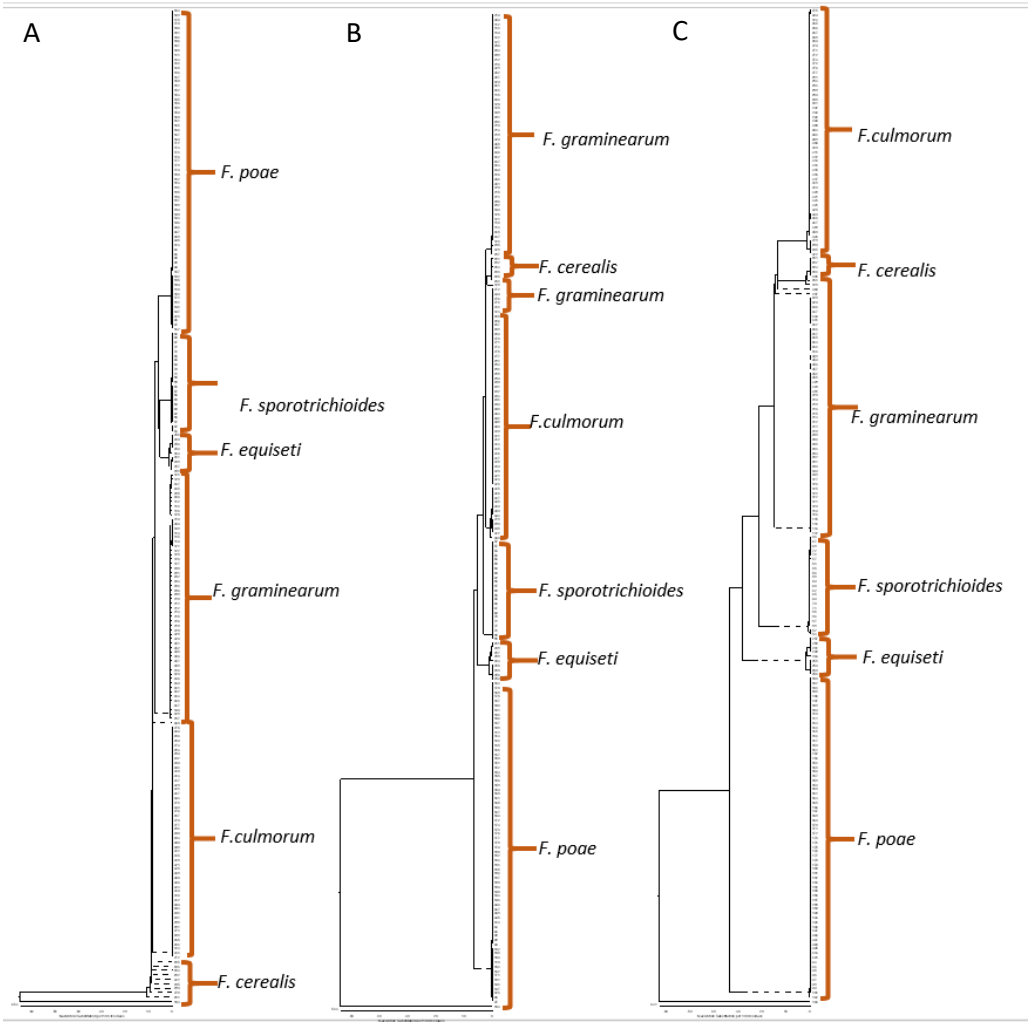

Figure 3: SDHD *trees Neighbor-Joining algorithm, A: whole sequence, B: coding sequence, C: non-coding sequence*

3.3.2. *In vitro* sensitivity of five *Fusarium* species

Ten SDHIs were tested *in vitro* on 5 *Fusarium* species (6 isolates per species) in comparison to prothioconazole-desthio, the active ingredient of the fungicide proline (Bayer, DMI), which is already used in agriculture against *Fusarium* [73]. Looking at differences species x fungicide by using an average of the 6 tested isolates per species (Fig. 4), different sensitivities between individual SDHI fungicides occurred from <0.1ppm (pydiflumetofen on *F. poae*) to 20 ppm (fluopyram on *F. graminearum*). SDHI fungicides could be divided in three groups of sensitivities. A first group had a very low efficacy on *Fusarium* species including fluopyram, penflufen, bixafen and isopyrazam, where growth was not inhibited for 4/5 *Fusarium* species (EC_{50}>3 ppm). A second group with fungicides having at least partial efficacy on 2 or more selected species, with boscalid, penthiopyrad, fluxapyroxad, sedaxane and

benzovindiflupyr. The third group included fungicides with a high efficacy on all *Fusarium* species, included pydiflumetofen as the only SDHI fungicide, and prothioconazole-desthio but which had a lower efficacy on *F. equiseti* (0.6 ppm), *F. culmorum* (1.5 ppm).

Sensitivities within a same species was also variable (Fig. 5) as expected from the choice of isolates used in this panel. Differences in sensitivity was visible with variability in standard deviation from 0.2 to 9.8 for *F. graminearum*, 0.09 to 7.9 for *F. culmorum*, 0.05 to 8.3 for *F. poae*, 0.06 to 9.7 for *F. sporotrichioides* and 0.02 to 8.7 for *F. equiseti*. Interestingly, in each species there were isolates which could be regarded as more or less sensitive to a specific fungicide. Pydiflumetofen was the only SDHI able to control all isolates with an EC_{50} of approximately 1 ppm and below. The isolate CS-FU00284 (*F. culmorum*) had the lowest sensitivity to pydiflumetofen with EC_{50} 1.5 ppm. Very surprising in these results were an apparent lack of correlation for sensitivity to different SDHI fungicides. This finding will require a more in-depth analysis of the data to better understand the results.

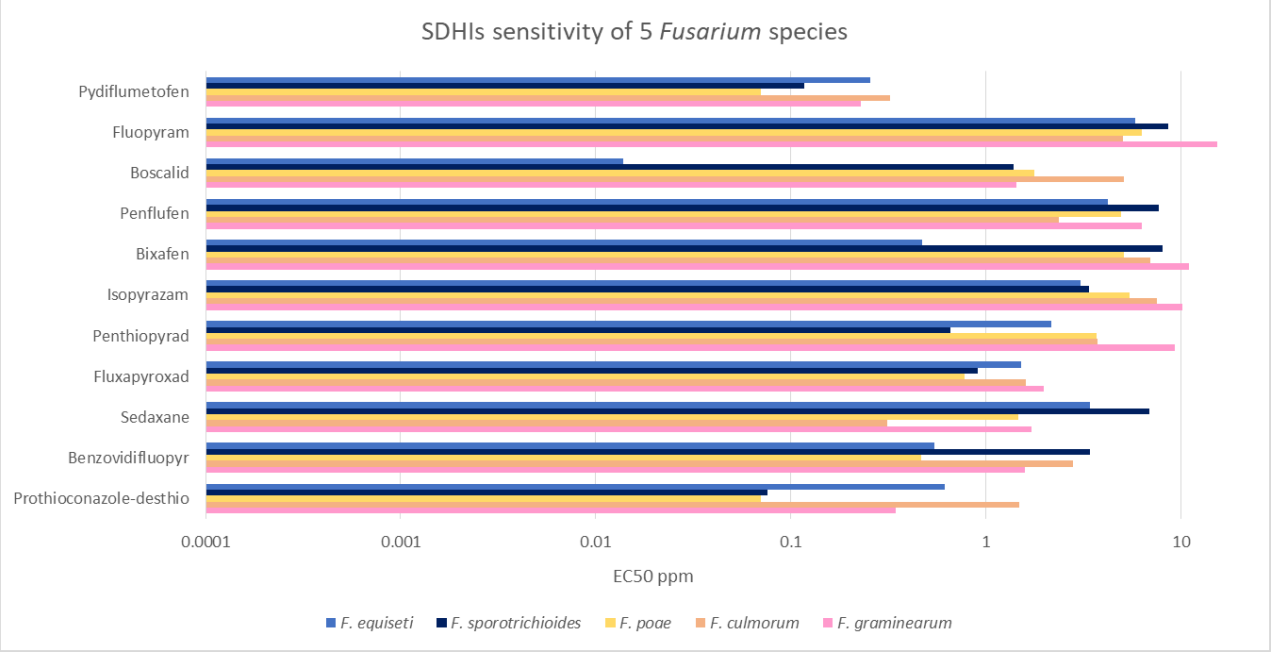

Figure 4: In vitro *sensitivity of 5* Fusarium *species and 11 fungicides. Abscise is the* EC_{50} *value in ppm (log (10)).*

F. *graminearum*	Pydiflumetofen	Benzovidifluopyr	Sedaxane	Fluxapyroxad	Penthiopyrad	Isopyrazam	Bixafen	Penflufen	Boscalid	Fluopyram	Prothioconazole desthio
CS-FU00125	6.3E-02	2.4E+00	3.5E-04	1.6E+00	2.0E+01	7.0E+00	1.6E+01	2.3E+00	5.0E+00	2.0E+01	1.1E-01
CS-FU00199	6.3E-02	3.0E+00	8.4E+00	6.6E+00	1.4E+01	2.0E+01	2.0E+01	1.1E-03	3.6E-05	2.0E+01	8.2E-02
CS-FU00128	9.4E-02	3.2E+00	3.1E-01	1.1E+00	2.0E+01	2.0E+01	2.0E+01	1.0E+01	1.4E-01	1.0E+01	4.4E-02
CS-FU00218	1.4E-01	8.6E-01	2.3E-02	8.0E-03	2.0E-04	6.1E+00	2.2E-07	2.0E+01	1.9E-01	1.6E+01	1.3E-01
CS-FU00352	4.6E-01	6.4E-02	3.7E-04	2.3E-02	2.8E-03	7.2E+00	1.0E+01	1.3E+00	3.0E-03	2.0E+01	6.5E-02
CS-FU00437	5.4E-01	3.3E-03	1.6E+00	2.5E+00	1.8E+00	3.0E-01	6.4E-07	4.1E+00	3.3E+00	5.6E+00	1.6E+00
Standard deviation	0.2156	1.4629	3.3097	2.4742	9.8066	8.0718	9.2545	7.5841	2.1754	6.1580	0.6353
F. *culmorum*											
CS-FU00360	1.3E-02	1.1E+00	4.2E-01	1.2E+00	8.2E+00	4.0E+00	1.2E+01	3.2E+00	1.2E+01	1.3E+00	7.4E-02
CS-FU00357	4.5E-02	3.0E+00	1.5E-03	2.1E+00	1.7E+00	7.0E+00	2.0E+01	2.5E-02	1.5E+00	7.9E+00	1.2E-01
CS-FU00279	1.2E-01	2.8E-04	3.3E-04	7.3E-02	2.2E-02	2.0E+01	5.5E+00	1.3E-02	1.0E+01	2.0E+01	2.6E+00
CS-FU00271	2.1E-01	1.7E+00	1.5E-04	2.6E-04	1.0E+01	3.5E-01	3.3E+00	7.4E-07	7.0E+00	4.2E-01	6.7E-02
CS-FU00294	2.2E-01	8.6E-01	9.7E-01	6.3E+00	2.2E+00	1.1E+01	5.1E-01	1.1E+01	6.4E-05	4.5E-01	5.1E-02
CS-FU00284	1.3E+00	1.0E+01	4.8E-01	1.4E-02	1.3E-01	2.0E+00	6.6E-01	2.8E-02	1.5E-02	3.7E-02	6.0E+00
Standard deviation	5.1E-01	3.7E+00	3.9E-01	2.4E+00	4.3E+00	7.3E+00	7.6E+00	4.3E+00	5.3E+00	7.9E+00	2.4E+00
F. *poae*											
CS-FU00104	1.1E-02	1.7E-02	8.0E+00	6.1E-05	7.7E-02	1.0E+01	2.0E+01	7.9E-02	4.3E-06	1.4E+00	5.1E-02
CS-FU00159	2.4E-02	5.8E-01	8.4E-02	1.2E+00	1.6E+00	6.3E-01	3.9E-01	2.0E+00	5.3E-01	9.2E-01	6.0E-02
CS-FU00197	3.4E-02	3.1E-02	1.2E-01	3.7E-01	4.2E-01	1.1E-04	1.1E-06	3.9E-02	3.2E-03	1.2E+01	5.0E-02
CS-FU00160	7.8E-02	6.4E-01	1.1E-01	1.8E-02	1.1E-01	1.3E-04	2.5E-02	1.8E+00	1.4E-04	8.8E-01	1.9E-01
CS-FU00110	8.9E-02	8.5E-02	4.1E-01	2.9E+00	2.0E+01	1.4E+01	1.0E+01	1.6E+01	1.0E+01	1.0E+01	4.7E-02
CS-FU00367	1.9E-01	1.5E+00	2.9E-02	1.8E-01	2.4E-06	7.7E+00	1.9E-01	1.0E+01	7.8E-02	1.3E+01	2.7E-02
Standard deviation	6.4E-02	5.6E-01	3.2E+00	1.1E+00	8.0E+00	6.1E+00	8.3E+00	6.5E+00	4.0E+00	5.8E+00	5.7E-02
F. *sporotrichioides*											
CS-FU00420	1.8E-02	8.9E-03	2.3E-01	2.1E+00	2.4E-02	1.6E-02	6.1E-01	6.8E-02	6.9E-01	1.9E-01	6.3E-02
CS-FU00056	1.0E-01	5.8E-02	7.6E-01	7.3E-01	1.3E+00	1.5E-01	2.0E+01	1.1E+00	7.0E-01	4.4E+00	4.7E-02
CS-FU00066	1.2E-01	2.0E+01	2.0E+01	9.5E-03	1.1E-01	2.0E+01	7.3E+00	3.5E+00	5.6E+00	2.9E+00	4.4E-02
CS-FU00074	1.2E-01	1.8E-01	1.0E+01	1.4E-01	2.5E+00	1.9E-02	4.8E-02	1.5E+00	4.8E-02	2.0E+01	5.0E-02
CS-FU00373	1.4E-01	1.1E-01	3.4E-02	4.0E-04	3.8E-03	9.3E-02	7.6E-03	2.0E+01	1.2E+00	4.5E+00	2.0E-01
CS-FU00069	2.1E-01	1.8E-01	1.0E+01	2.5E+00	8.5E-03	1.9E-03	2.0E+01	2.0E+01	6.1E-03	2.0E+01	4.4E-02
Standard deviation	6.3E-02	8.1E+00	8.0E+00	1.1E+00	1.0E+00	8.1E+00	9.7E+00	9.6E+00	2.1E+00	8.9E+00	6.4E-02
F. *equiseti*											
CS-FU00536	6.9E-04	3.5E-02	2.1E-04	8.8E+00	5.5E-04	3.9E-01	4.4E-07	2.1E-01	4.2E-03	3.3E-02	1.7E+00
CS-FU00493	6.5E-02	1.1E-01	3.4E-01	4.6E-02	2.9E+00	4.6E+00	2.0E-01	1.0E+01	5.9E-02	5.0E+00	4.1E-02
CS-FU00527	1.3E-01	1.9E-01	8.7E-03	1.2E-04	5.0E-04	2.4E-01	6.8E-08	3.3E+00	1.6E-04	1.0E+01	1.9E-01
CS-FU00510	2.0E-01	1.3E+00	3.3E-04	4.6E-03	2.3E+00	1.2E+01	2.4E-02	6.2E-01	9.1E-04	1.2E-03	1.5E+00
CS-FU00518	2.1E-01	1.7E-01	1.4E-01	9.7E-02	6.6E+00	3.5E-04	2.6E+00	1.0E+01	1.8E-02	2.0E+01	2.7E-01
CS-FU00521	9.3E-01	1.5E+00	2.0E+01	9.0E-02	1.2E+00	1.1E+00	4.9E-07	7.3E-01	8.0E-04	9.9E-03	2.3E-02
Standard deviation	3.4E-01	6.5E-01	8.1E+00	3.6E+00	2.5E+00	4.7E+00	1.0E+00	4.8E+00	2.3E-02	8.0E+00	7.6E-01

Figure 5: in vitro *EC_{50} values of* Fusarium *isolates. The color scale shows in dark green the very sensitive isolates (<0.01 ppm) the mid sensitive to less sensitive and the resistant in red (>1.6ppm). Rows are the EC50 values of each isolate for each fungicide.*

3.3.3. Dose response to pydiflumetofen *in planta*

A dose-response assay on plants was done on all isolates used in the *in vitro* assay to show if the differences in the sensitivities seen in the *in vitro* assays were also visible *in planta* or not. 14 days after inoculation plants were assessed by estimating the percentage of infection on the ears. The percentage of disease control (DC) versus untreated controls was then calculated. The experiments were conducted in duplicate, with an independent set of plants infected at a different time with the same isolates.

Tested isolates for *F. graminearum* (Fig. 6 A, B) showed good symptoms of infection for both assays (between 100% and 30% of infection). Dose response of fungicides was visible with higher control in plants treated with higher fungicide concentrations for all isolates excepted for CS-FU00352 with a very flat dose response in both assays, for both fungicides tested. Statistical analysis for assay 1 did not show significant differences between the two fungicides for none of the isolates. However, for isolates CS-FU00125, CS-FU00128, C CS-FU00352 and CS-F00437 the trend of a better control for APN compared to proline was visible. In contrast, for isolate CS-FU00218 proline seemed more effective, and for CS-F00199 both fungicides were equal. In assay 2, isolates CS-FU00125 and CS-F00218 showed significant differences in favor of APN, while again differences were non-significative for all other isolates). A trend in favor of APN was visible in the isolates CS-F00199 and CS-F00437, and no differences in the other two isolates. Overall, the results on disease control from greenhouse experiments were found very variable from assay to assay, and isolates differentiated based on *in-vitro* EC_{50} did not behave obviously different *in-planta* based on their disease control from Adepidyn.

F. culmorum (Fig. 7 A, B) isolates resulted in good plant infection (30-100% infection rate) and dose response was visible for most isolates. Overall, the efficacy against *F. culmorum* was lower as compared to *F. graminearum*. In assay 1 plants infected with isolates CS-FU00271 and CS-FU00279 treated with proline 200 g/ha showed a trend for higher efficacy compared to plants treated with APN 200 g/ha (13% and 10%, not significant). On the other hand, two other isolates showed a trend in favor of APN, and two were equal. In assay 2 there were 3 isolates in favor of APN, two neutral, and one in favor of proline (significant at lower rates). In this assay, two isolates developed very strong infection pressure resulting in overall low fungicide activity (CS-FU00270 and CS-FU00357).

Tested isolates for *F. poae* (Fig. 8 A, B) infected plants (5-100%) and dose response was visible in assay 2, however in assay 1 the lowest concentration was sometimes higher than the highest concentration. In assay 1 plants infected with isolates CS-FU00159 did not show any symptoms and there was no statistics possible on plants infected with CS-FU00197 and CS-FU00367. There was no significant difference in plants inoculated with isolates CS-FU00104 and higher efficacy for APN vs

proline for CS-FU00110 at low rates. Plants infected with CS-FU00160 and treated with APN 200 g/ha showed higher efficacy compared to plants treated with proline 200 g/ha (not significant). In assay 2 there was no significant difference in plants inoculated with isolates CS-FU00104, CS-FU00159, CS-FU00197. Plants infected with CS-FU00110 and CS-FU00367 showed an advantage in favor of APN, while isolate CS-FU00160 was more sensitive to proline at low rates.

Tested isolates for *F. sporotrichioides* (Fig. 9 A, B) infected plants well (25-60%), the results in assay 1 and assay 2 seemed most reproducible. A dose response was visible for most isolates. In assay 1 plants infected with isolates CS-FU00066, CS-FU00074 and CS-FU00420 did not show significant differences. There was no disease control on plants infected with CS-FU00056 and treated with APN, while proline retained activity. Plants infected with CS-FU00373 and treated with APN 200 g/ha showed higher efficacy compared to plants treated with Proline 200 g/ha (60% to 50% DC). In assay 2 there was no statistics possible on plants infected with CS-FU00373 and CS-FU00420 (all treatments were similar). Plants infected with isolates CS-FU00066, CS-FU00069 and CS-FU00074 and treated with both fungicides did not show any significant differences between the treatments. Plants infected with CS-FU00056 and treated with proline 200 g/ha showed higher efficacy compared to plants treated with APN 200 g/ha (80% DC to 70% DC).

Tested isolates for *F. equiseti* (Fig. 10 A, B) infected plants reproducibly in both assays (40-70%) and dose response was visible for most isolates. Both assays were very similar with isolates CS-FU00518, CS-FU00521 and CS-FU00536 and treated with both fungicides did not show any significant differences between the treatments. Plants infected with isolates CS-FU00493 (significant), CS-FU00510 and CS-FU00527 and treated with APN 200 g/ha showed higher DC (85%, 88%, 95%) compared to proline 200 ppm (65%, 82% and 68%).

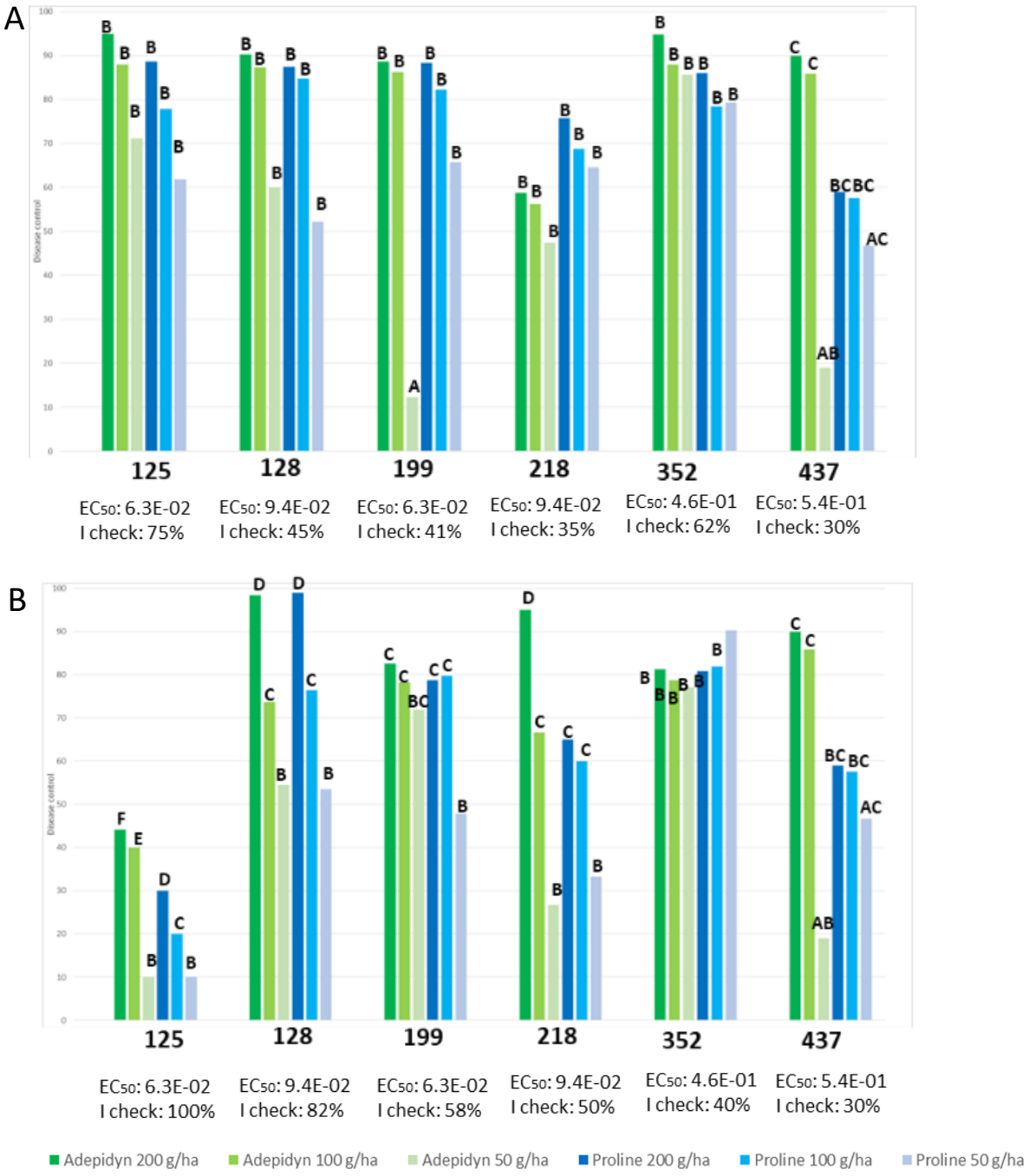

Figure 6: A, B, two independent repetitions: Disease control of F. graminearum *with different fungicide concentrations. Letters on the graphs correspond to the statistical differences. Under each isolate number* EC_{50} *values* in vitro *and percentage of infection on the ears of the plants inoculated.*

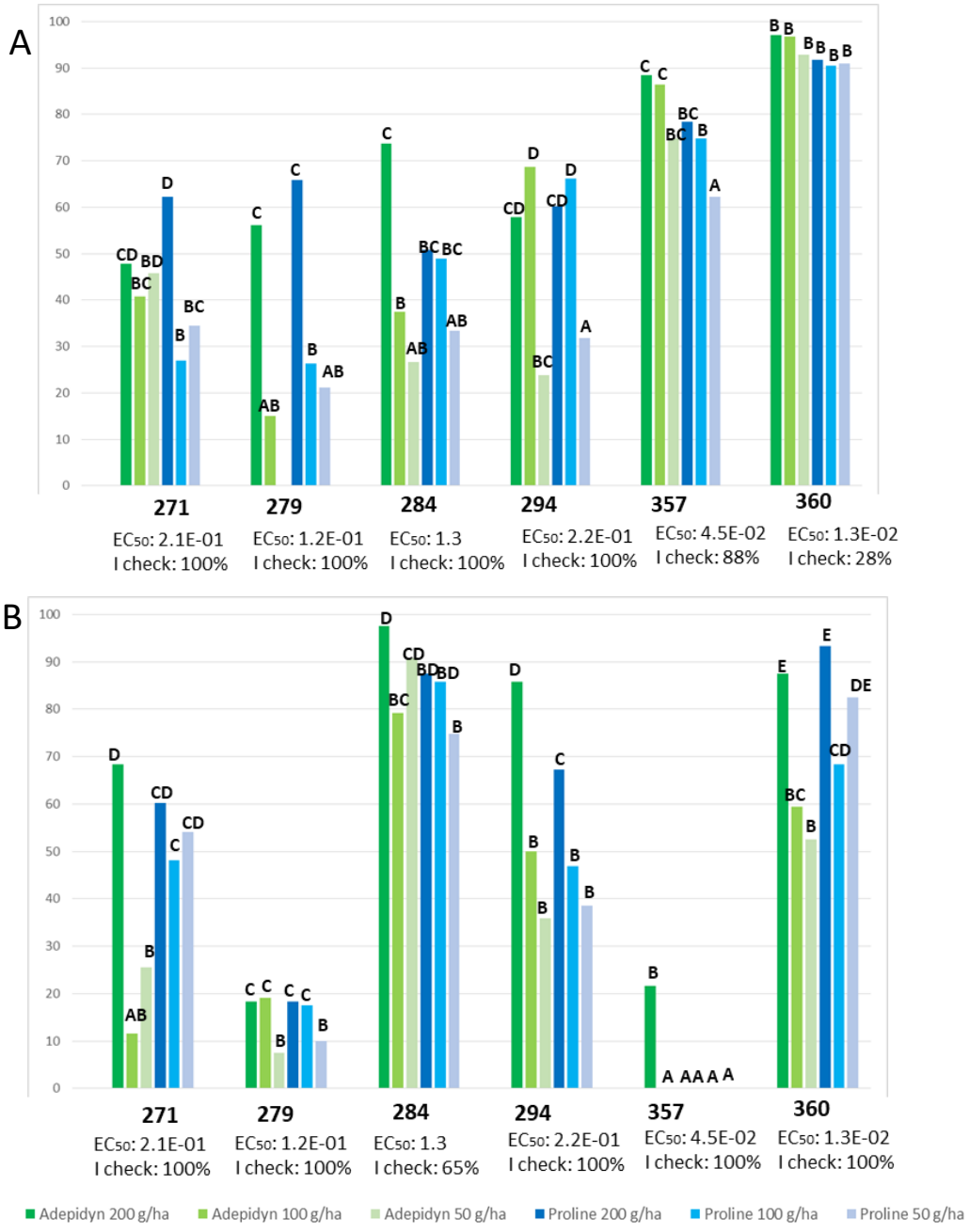

Figure 7: A, B two independent repetitions: Disease control of F. culmorum *with different fungicide concentrations. Letters on the graphs correspond to the statistical differences. Under each isolate number* EC_{50} *values* in vitro *and percentage of infection on the ears of the plants inoculated.*

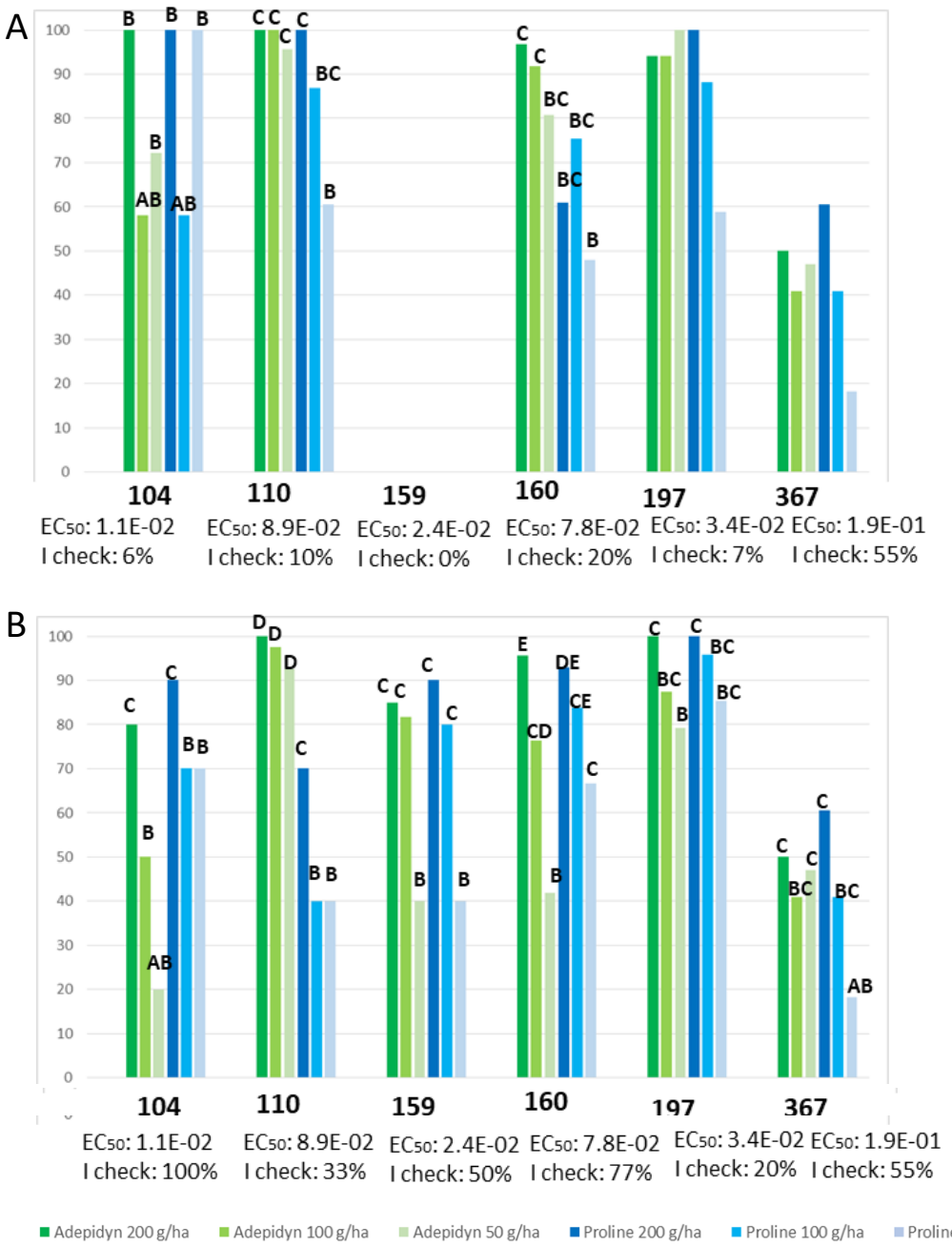

Figure 8: A, B two independent repetitions: Disease control of F. poae *with different fungicide concentrations. Letters on the graphs correspond to the statistical differences. Under each isolate number* EC_{50} *values* in vitro *and percentage of infection on the ears of the plants inoculated.*

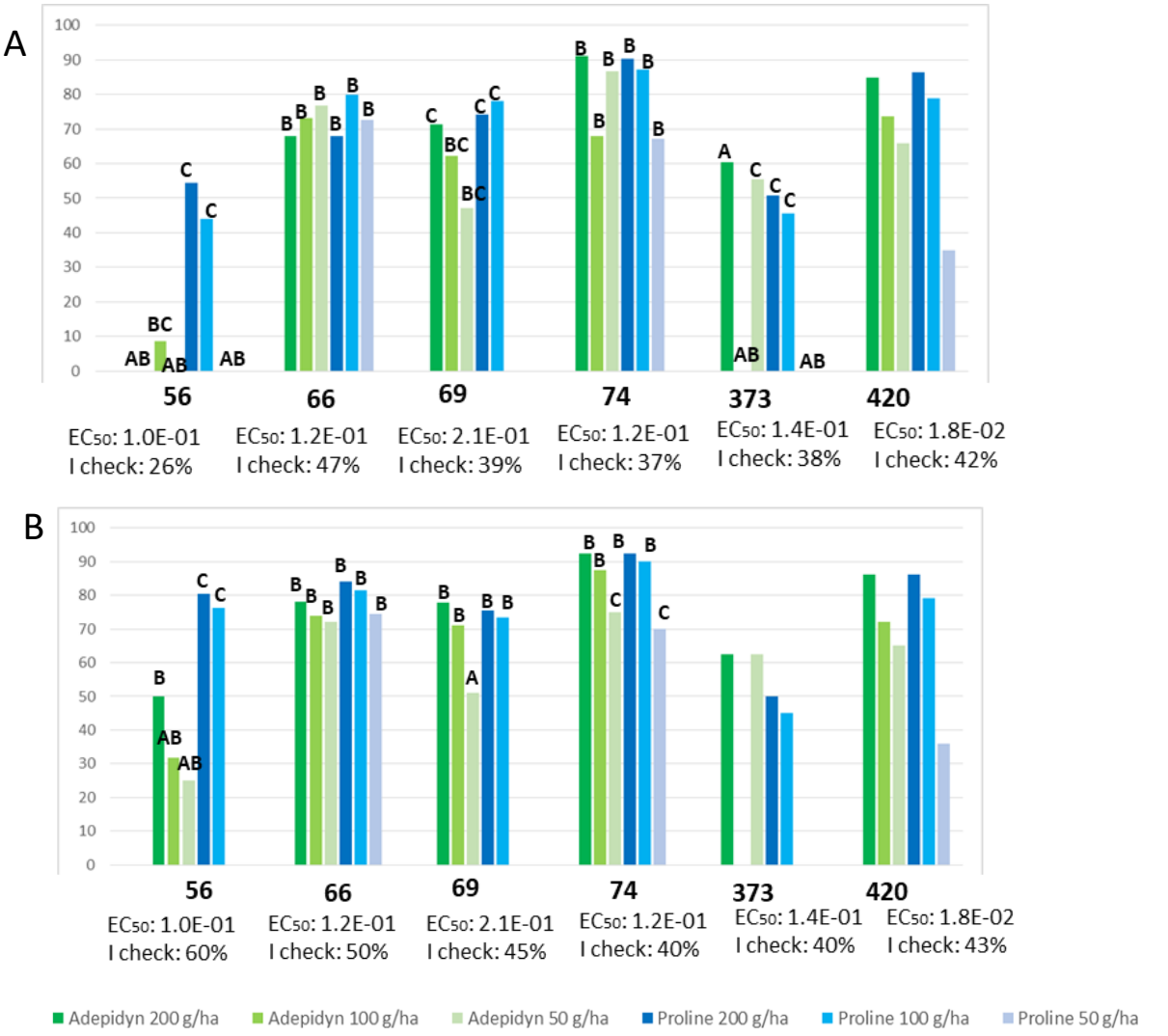

Figure 9: A, B two independent repetitions: Disease control of F. sporotrichioides *with different fungicide concentrations. Letters on the graphs correspond to the statistical differences. Under each isolate number* EC_{50} *values* in vitro *and percentage of infection on the ears of the plants inoculated.*

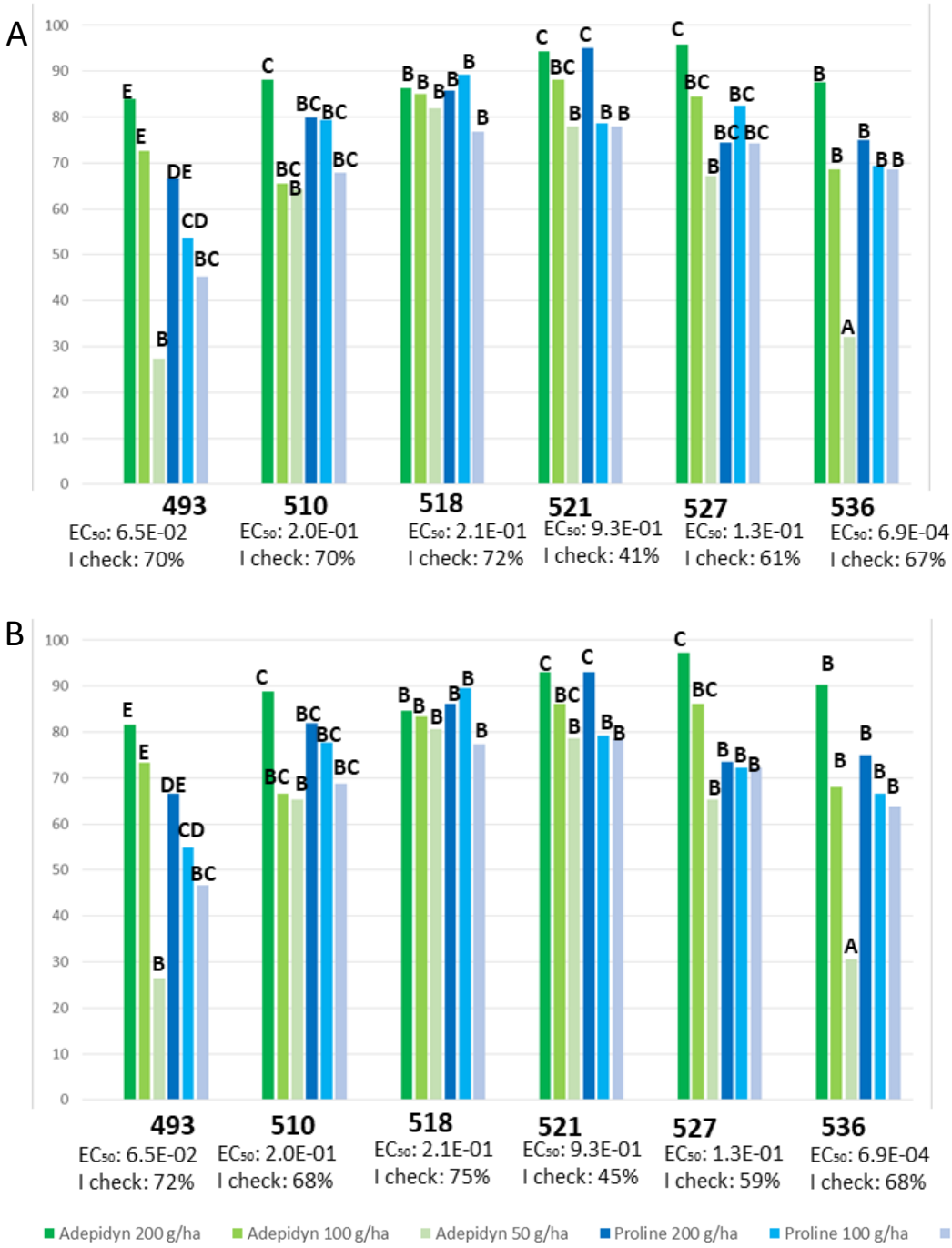

Figure 10: A, B two independent repetitions: Disease control of F. equiseti *with different fungicide concentrations. Letters on the graphs correspond to the statistical differences. Under each isolate number* EC_{50} *values* in vitro *and percentage of infection on the ears of the plants inoculated.*

3.4. Discussion

Actually SDHI fungicides are important for the management of several diseases in a wide range of pathogens and crops [95]. These molecules bind to the ubiquinone binding site of the mitochondrial complex II and inhibit fungal respiration [96]. Over years of using SDHI fungicides many cases of resistance have been reported as in *Zymoseptoria tritici* to fluopyram [81] or *Alternaria alternata* to boscalid [97]. Knowledge about the genetic factors influencing resistant populations is an important step to improve the use of SDHI fungicides [96]. Molecular mechanisms of resistance have already been studied and it was shown that the same mutation of the conserved histidine residue determines resistance to carboxamide but also to boscalid [97]. Lab mutants resistant to SDHIs in *Botrytis cinereal* generated by site directed mutagenesis of the *SDHB* gene, showed that every mutation generated, conferred resistance to boscalid and cross-resistance to other SDHIs [79]. Molecular studies showed that the mutations are very close to the ubiquinone binding site and then avoid the active ingredient of the fungicide to bind to the SDH enzyme [98].

In this study it was shown that among *Fusarium* species and among different isolates of the same species there are differences in SDHI fungicide sensitivity. Cross resistance was shown with several species resistant to boscalid, fluopyram, penflufen or isopyrazam. Molecular mechanisms of resistance to SDHIs are also a reality in *Fusarium* species. However, sensitivity to pydiflumetofen was shown and even if species were resistant against other tested fungicides no resistance was found to pydiflumetofen. That was also shown in China on a panel of 116 *F. asiaticum* [99], where the fungicide had strong inhibition effect on mycelial growth and conidia germination even at very low concentrations.

In this study we included in our panel an in-house lab-mutant resistant to pydiflumetofen (Fig. 4, Appendix). I showed *in vitro* that even at high pydiflumetofen concentrations the isolate was able to keep on growing (Fig. 4A, Appendix) and was not more tolerant to solatenol, which confirms again that cross resistance do not occur automatically for particular mutations. *In vitro* data for this isolate showed EC_{50} value of 1.7 ppm compared to the WT 0.007 ppm (Fig. 4B, Appendix). We can assume that isolates having an EC_{50} value higher than 1.5 ppm are considered to be adapted. No other adapted isolate was found in the panel. Isolate CS-FU00284 showed EC_{50} value of 1.3 ppm but if the protein sequence of *SDH* genes were compared to the lab mutant, no mutation was found whereas the lab mutant had two mutations in its protein sequence on *SDHB* gene (Fig. 4C, Appendix).

Differences in species sensitivities to SDHIs might be explained th rough the molecular data we generated. Phylogeny of the species on the nucleotide sequence showed a conserved relation within a same species. Species kept their clusters that means there is variability among species in the *SDH*

genes. However not all SDH subunits could be sequenced which indicates further efforts will be necessary to amplify those sequences, e.g. by amplification of 5' and 3' ends of mRNA using RACE and design of new species-specific primer sets. The availability of additional *Fusarium* species genomes could be helpful.

Missing in this work due to limited time available is a more thorough analysis of EC_{50} values for APN and solatenol vs the genotype of the respective *SDH* gene subunits. Clustering sequences with similar EC_{50} values may reveal sequence differences important for sensitivity to either APN or solatenol. Alternatively, it is also conceivable the differences observed are not mainly driven by the SDH protein complex but might also involve other as yet not identified factors in *Fusarium*, that are variable in different isolates and different species.

The large differences observed for EC_{50} values *in vitro* (> 10x differences in sensitivity) did apparently not strongly influence the *in-planta* sensitivity towards APN. This is reassuring, as isolates found least sensitive in the *in-vitro* work are equally well controlled *in-planta*. This could indicate that for *Fusarium*, as discussed above, variability other than on the SDH enzyme might be more relevant for *in-vitro* work, but not relevant (or less relevant) for sensitivity on the whole plant. A follow-up on this would require infection of field plots with selected *Fusarium* isolates with differences in APN sensitivity. However, ethical considerations preclude such work, at least at the moment, as no shift of APN sensitivity in *Fusarium* field trials has been reported to date.

The FRAC (Fungicide Resistance Action Committee) set the resistance risk of SDHI fungicides as medium to high level because of their single site mode of action. Pydiflumetofen provided a strong inhibition potency to other fungal pathogens [100], [101] which indicates that its binding domain in the SDH complex might be highly adapted to bind to the SDH enzyme. *In planta* data showed very good control of *Fusarium* and the same was shown in another study with *F. asiaticum* [99]. Further assays were done in chapter 4 to find the right application timing of pydiflumetofen, available under Adepidyn™ [84] .

4. Application timing on wheat in the greenhouse

4.1 Introduction

Fusarium head blight (FHB) on wheat (Triticum sp.) is caused by species of the fungus *Fusarium* and results in high yield losses due to mycotoxin production [102], under disease favorable conditions, and by impacting the grain quality in reduction of albumin and gluten proteins [10]. Mycotoxin lead to severe diseases in human and animals if eaten [50]. During anthesis, the florets of the wheat are opening to release the pollen and make an opening for the fungal pathogens which can infect plants [29]. The infection occur when warm and wet weather conditions coincide with the flowering time of wheat [31]. Spores germinate after a few hours in plant contact [33] and during the first infection stages the infection is symptomless [31]. The pathogen spreads from spikelet to spikelet through vascular tissues and rachis [15]. Out of the more than 500 genes that are expressed during the infection stages of the pathogen [33] toxin synthesis genes expressed during early stages of infection were found to be responsible for secondary metabolite production [33].

Besides agricultural practices including crop rotation, tillage and optimal fertilization [18] and disease tolerant varieties, it remains difficult to control FHB and fungicides remain the most effective way to manage the disease [63]. Disease severity on the spikes is lower at later infection timings [103] and later fungicide applications can reduce mycotoxin contamination [69] and would allow farmers to apply fungicides after rain events [104]. In contrast application of prothioconazole, a demethylation inhibitor fungicide (DMI) reduced FHB and deoxynivalenol level if applied before head emergence [73].

It was shown in previous studies (Chapters 2 and 3), that a novel developed SDHI fungicide - Adepidyn™ (pydiflumetofen, Syngenta) [84] showed very good *in vitro* efficacy on *Fusarium* species. Previous studies showed also a decrease in symptoms and mycotoxin contamination in treated plants. The same was reported for field assay performed in China on *F. asiaticum* [99]. However, application timing of pydiflumetofen on wheat remains unclear. The aim of this study was to identify the optimal application timing of pydiflumetofen compared to proline (prothioconazole, Bayer CropScience), to reduce infection and mycotoxin production. Therefore, studies were performed in the greenhouse on wheat plants and long preventive and long curative application timings were chosen on *F. graminearum*, *F. culmorum* and *F. poae*. Preventive treatments mean that plants were treated before infection occurred and curative treatments mean that plants were treated after infection. Putatively preventive and curative application regime could influence the FHB complex and ables its composition. Mycotoxin analysis were performed for each application timing.

4.2 Material and Methods

All assays were performed using wheat plants. Spring wheat seeds of the variety Monsun were seeded at 5 seeds per pot (mixed earth 2 g of fertilizer per L of soil and (2-chloroethyl) trimethylammonium chloride (CCC) treatment 4 mL/L)). After 2 weeks, seedlings were reduced by snatching weakest plant to 4 seedlings per pot. After 9- and 11-weeks plants were trimmed during growing to get only 4 main ears per pot. Plants were used for the assay at full flowering after approximately 12 weeks. Greenhouse conditions were 19°C night for 12 hours, 21°C day for 12 hours, 80%RH and plants were irrigated every day. Each assay was performed two to three times at independent timings. Fungal isolates (Table 1) were grown on PDA (potato dextrose agar, 39 g/L) plates for 12 days at 65% HR, 12 hours dark and 12 hours UV light. For each assay at full maturity, ears were collected for toxin analysis on grains.

Table 1: Fusarium *isolates used for* in planta *infection assays*

	Isolates	Country of origin	Year of isolation	Spore suspension (sp/ml)
F. graminearum	K6139/CS-FU00123	France	2002	200‘ 000
	K6934/CS-FU00124	Germany	2013	
	K6935/CS-FU00125	Germany	2012	
F. culmorum	K6936/ CS-FU00293	Germany	2013	200‘ 000
	K6937/ CS-FU00326	Germany	2013	
	K6938/ CS-FU00327	Germany	2012	
F. poae	K8029/ CS-FU00102	France	2015	400‘ 000
	K8031/ CS-FU00162	Germany	2015	
	K8039/ CS-FU00184	Germany	2010	

4.2.1. Long curative assay

At full flowering (12 weeks), ears were inoculated using a pipett with 10 µl of a *F. graminearum* spore suspension (200 000 sp/mL) into one marked spikelet of each ear. The spore suspension is composing by three different *F. graminearum* isolates (K6139, K6934, and K6935). After the infection, plants were put in a climatic chamber at 19°C, complete dark and 100%RH for incubation. After 2 days plants were put in a greenhouse at 19°C night for 12 hours, 21°C day for 12 hours, 80% RH and were irrigated every day. After 3, 5, 7, 10 and 12 days after inoculation (curative applications), plants were treated (adepidyn at 200 g/ha, 100g/ha 50 g/ha, and proline at 200 g/ha, 100 g/ha 50 g/ha, Table 2), using a track sprayer (Caromatic Swiss technology, 2006) machine. Ten days and 14 days after each application, infection was assessed in percentage of symptoms visible on each ear (symptoms are the

area of dark/brownish spots visible on the infected ear). Data from the inoculated plots were subjected to analysis of variance using the Syngenta in-house statistical package Acsapwin. The terms in the statistical model were treatment and block. Prior to analysis, the percentages were arcsin-transformed, i.e. y=arcsin√(x/100) for normalization. The statistical significance of the overall effect of treatment was assessed via an F-test. In cases where the F-test was significant at the customary 5% probability level (i.e. F-test probability <5%), the significance of differences between specific treatments, including the inoculated check, was assessed using the LSD (Least Significant Difference) method. Means on the transformed scale that differed by more than the relevant LSD were considered significantly different at the customary 5% probability level, providing evidence of a genuine difference between the two treatments in question. Differences that were smaller than the relevant LSD were interpreted as no greater than expected under random variation and did therefore not provide convincing evidence of a genuine difference between the two treatments in question. The outcome of all possible treatment comparisons are summarized by letters such that no letter in common reflect are significant differences.

4.2.2. Long preventive assay

At full flowering (12 weeks) plants were treated (Table 2). At 1, 3, 5, 7 and 9 days after application plants were inoculated (preventive application) with a spore suspension (Table 1) using a paint brush at 1.5 mbar. After each infection, plants were put at 1°C, complete dark and 100%RH. After 2 days plants were put in a greenhouse at 19°C night for 12 hours, 21°C day for 12 hours, 80%RH and were irrigated every day. After 10 days and 14 days the infection was assessed in percentage of symptoms visible on each ear. Data from the inoculated plots were subjected to analysis of variance using the Syngenta in-house package Acsapwin. The terms in the statistical model were treatment and block and analysis was done as above.

Table 2: treatments and number of pots (4 ears per pot) used for each assay

	F. graminearum long curative			*F. graminearum* long preventive			*F. culmorum* long preventive		*F. poae* long preventive	
	rep-1	rep-2	rep-3	rep-1	rep-2	rep-3	rep-1	rep-2	rep-1	rep-2
treatments	nb of pots									
Check untreated, uninoculated	3	3	3	3	3	3	3	3	3	3
Check inoculated	3	3	3	3	3	3	3	3	3	3
Adepidyn 50g/ha		3	3			3	3	3	3	3
Adepidyn 100g/ha		3	3		3	3	3	3	3	3
Adepidyn 200g/ha	3	3	3	3	3	3	3	3	3	3
Proline 50g/ha		3	3			3	3	3	3	3
Proline 100g/ ha		3	3		3	3	3	3	3	3
Proline 200g/ha	3	3	3	3	3	3	3	3	3	3

4.3 Results

4.3.1. Long curative

All ears were inoculated by a single spikelet method at the same time but treated at 3, 5, 7, 10 and 12 days after inoculation (dai). The assay was repeated three times and statistics were done on each of the assay (Table 3). Infection occurred at each assay, as the check inoculated ears had between 19% up to 65% symptoms. All treatment reduced symptoms appearance for all treatments, except proline 50 g/ha.

Plants treated at 3 dai showed no significant differences between APN 200 g/ha and Proline (PTZ) 200 g/ha (p=0.05). For rep-3 treatments with APN 100 g/ha showed no significant difference to PTZ 200 g/ha. Looking on disease control (DC) over all three repetitions APN 200 g/ha DC is identical to PTZ 200 g/ha (each 59%). DC was better for APN 100 g/ha and APN 50 g/ha (38% and 45%, respectively) than for PTZ 100 g/ha and absence of DC for PTZ 50 g/ha (30% and absent, respectively).

At 5 dai there was no significant differences between the treatments in rep-1 and rep-3 and statistics could not be done on rep-2 (p=0.05). However, the trend in rep-2 and rep-3 of better efficacy of APN 200 g/ha was detected (9 to 19% in rep-2 and 9 to 14% in rep-3). A better DC was shown with APN treatments (between 60 and 62%) than for PTZ treatments (30-49%).

At 7 dai significant differences in the treatments were shown in rep-1 with 22% of infection for APN 200 g/ha and 38% for PTZ 200 g/ha. For rep-2 and rep-3 no statistical analysis could be done, however, rep-2 showed a trend of better efficacy of APN 200 g/ha (10% symptoms to 19% for proline

200 g/ha) and seems to be more efficient on disease control than the other treatments, even on symptoms. APN 200 g/ha is still here the best concentration for disease control. Otherwise DC showed also better efficacy for APN 200 g/ha (58% to 38% for Proline 200 g/ha), for APN 100 g/ha (39% to 36% for PTZ 100 g/ha) but not for APN 50 g/ha (38% to 61% PTZ 50 g/ha).

At 10 dai rep-1 showed no significant differences between treatments but in rep-2 APN was significantly better at all rates (11 to 13%) than PTZ (16-23%). Rep-3 showed a trend of better efficacy with PTZ 200 g/ha (13% symptoms to 42% for APN 200g/ha). DC showed better efficacy for Proline at the highest rate (37% DC to 24% for APN) but not at 100 g/ha (63% DC for APN to 47% for PTZ).

At 12 dai there was a significant better efficacy of APN 200g/ha (39% of symptoms) compared to PTZ (52%) and the trend of better efficacy of APN 200 g/ha was visible in rep-2 (31% of symptoms to 61% for Proline 200 g/ha). Otherwise in rep-3 PTZ 200 g/ha has a significant better efficacy (2% of symptoms) compared to APN 200 g/ha (10%) but was identic to APN 100 g/ha. DC is higher for APN 200 g/ha (72%) than for PTZ (33%).

Disease control with APN 200 g/ha only decreased at 10 dai (24%) but was between 53% and 62% over time whereas DC with PTZ decreased with a later application.

Mycotoxin analysis (Fig. 1) showed high DON amounts in check inoculated grains (more than 11500 µg/g). At each time point grains treated with APN 200 g/ha and 100 g/ha showed a stronger reduction in DON amount if compared to those treated with PTZ 200 g/ha and 100 g/ha. In tendency DON amount were higher in more curative situations for APN.

If DC was compared to DON amounts, results showed an increasing mycotoxin level if DC decreased for both APN 200 g/ha and PTZ 200 g/ha.

Table 3: Results of long curative assay with F. graminearum*, Letters represent statistical differences between the treatments*

	Symptoms of the disease on the ears (%)							
3 DAI	rep-1	Letter	rep-2	Letter	rep-3	Letter	Ø % symptoms	Disease control
Check untreated uninfected	0		0		0		0	
Check infected	19	A	28	A	24	A	24	
Adepidyn 200 g/ha	11	B	9	B	9	B	10	59
Adepidyn 100 g/ha			16	AB	13	B	15	38
Adepidyn 50 g/ha			11	B	15	AB	13	45
Proline 200 g/ha	9	B	9	B	11	B	10	59
Proline 100 g/ha			19	AB	14	AB	17	30
Proline 50 g/ha			24	A	24	A	24	-2
5DAI								
Check untreated uninfected	0		0		0		0	
Check infected	45	A	27		37	A	36	
Adepidyn 200 g/ha	23	B	9		9	B	14	62
Adepidyn 100 g/ha			16		13	B	14	60
Adepidyn 50 g/ha			12		17	B	15	60
Proline 200 g/ha	24	B	19		14	B	19	48
Proline 100 g/ha			22		15	B	18	49
Proline 50 g/ha			21		30	A	25	30
7 DAI								
Check untreated uninfected	0		0		0		0	
Check infected	37	A	38		55		43	
Adepidyn 200 g/ha	22	B	10		23		18	58
Adepidyn 100 g/ha			15		47		31	28
Adepidyn 50 g/ha			13		39		26	39
Proline 200 g/ha	38	A	19		23		27	38
Proline 100 g/ha			16		39		28	36
Proline 50 g/ha			9		25		17	61
10 DAI								
Check untreated uninfected	0		0		0		0	
Check infected	60	A	30	A	43		45	
Adepidyn 200 g/ha	49	B	11	C	42		34	24
Adepidyn 100 g/ha			11	C	21		16	63
Adepidyn 50 g/ha			13	C	27		20	55
Proline 200 g/ha	48	B	23	AB	13		28	37
Proline 100 g/ha			16	BC	31		24	47
Proline 50 g/ha			19	BC	21		20	55
12 DAI								
Check untreated uninfected	0		0		0		0	
Check infected	65	A	58		50	A	58	
Adepidyn 200 g/ha	39	B	31		10	BC	27	53
Adepidyn 100 g/ha			30		2	C	16	72
Adepidyn 50 g/ha			35		10	BC	22	62
Proline 200 g/ha	52	AB	61		2	C	38	33
Proline 100 g/ha			67		21	B	44	24
Proline 50 g/ha			54		9	BC	32	45

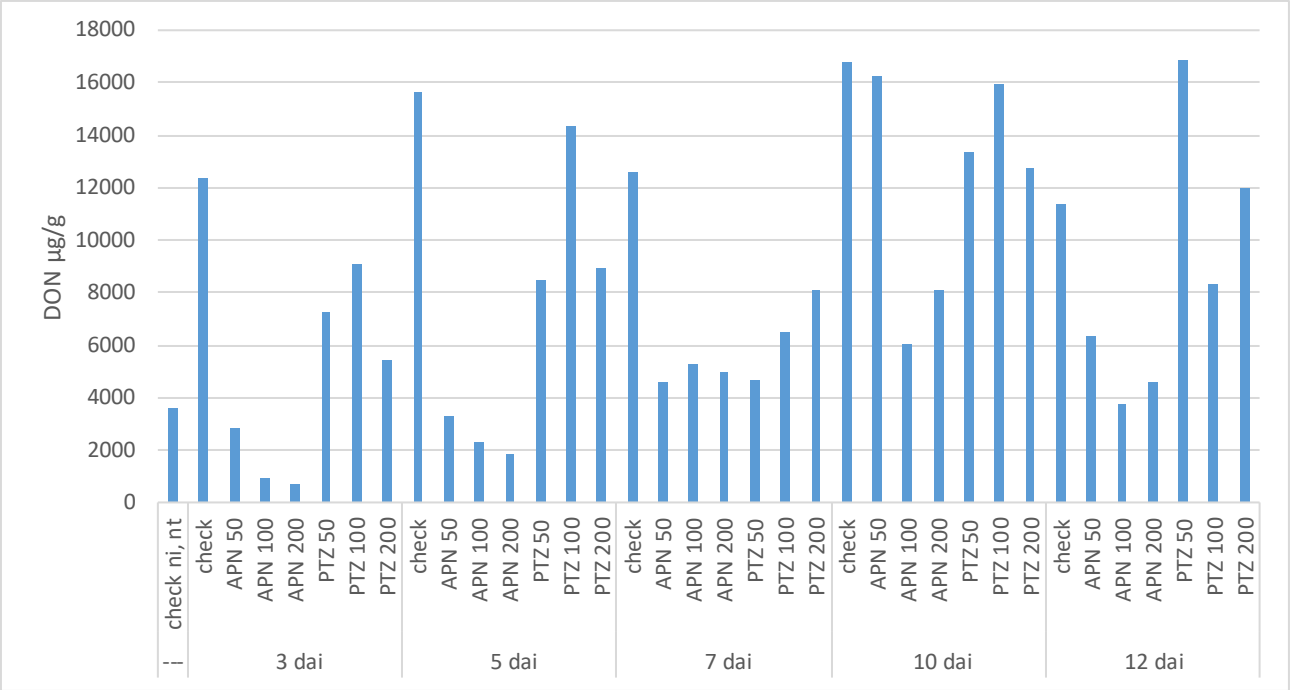

Figure 1: Mycotoxin amount produced by F. graminearum

4.3.2. Long preventive

4.3.2.1. Long preventive on F. graminearum

Plants were first treated and then inoculated at days 1, 3, 5, 7 and 9 after application (daa). The assay was repeated three times and statistics were done on each of the assays (Table 4). Mycotoxin amount in the grains was analyzed after complete maturation of the ears. Infection was prevalent for each assay, as the check inoculated ears had between 55% and 100% symptoms.

1 daa in rep-1 there was significant differences between the treatments with better efficacy on symptoms for APN 200 g/ha (1%) and PTZ 200 g/ha (13%). In rep-2 there were no differences between the treatments at 200 g/ha (19% for APN and 21% symptoms for PTZ). Again, the trend is here in rep-3 for a better efficacy of APN 200 g/ha (19% to 21% for PTZ) but the differences are not significant. Disease control was better for APN 200 g/ha than for PTZ 200 g/ha (89% to 80%).

At 3 daa there was a significant difference in rep-1 (APN 200 g/ha 3% to 17% of symptoms for PTZ 200 g/ha) and in rep-2 with Proline 23% and APN 200 g/ha 29%. No significant difference was noticed in rep-3 between the treatments even if the trend at all rates of APN was better (5-9% of symptoms) compared to PTZ (18-31%). DC was at 85% for APN 200 g/ha to 78% for PTZ 200 g/ha and APN 50 g/ha had 93% DC.

At 5, 7 and 9 daa the differences between the treatments were significant in all treatments with a reduction of the symptoms for ears treated with APN 200 g/ha: 5 daa: 9-18% against 25-66% for PTZ 200 g/ha and DC 82% for APN to 45% PTZ, 7daa: 0-12% against 23-78% for PTZ 200 g/ha and DC 89% for APN to 48% PTZ and at 9 daa: 7-20% against 42-53% PTZ 200 g/ha and DC 85% for APN to 50% PTZ.

At 1 daa mycotoxin amounts (Fig. 2) were reduced with APN 200 g/ha and 100 g/ha (less than 20 000 µg/g) compared to PTZ 200 g/ha, above 20 000 µg/g. At 3 daa mycotoxins were reduced with APN 100 g/ha (10 000 µg/g) compared to APN 200 g/ha and PTZ 200 g/ha (18 000 µg/g). At 5, 7 and 9 daa mycotoxins were reduced under 20 000 µg/g compared to PTZ reaching 40 000 µg/g.

If DC was compared to DON amounts, results showed no clear decreasing mycotoxin level if DC increased for both APN 200g/ha and PTZ 200 g/ha.

Table 4: Results of long preventive assay with F. graminearum. *Letters represent statistical differences between the treatments.*

	Symptoms of the disease on the ears (%)							
1 DAA	rep-1		rep-2		rep-3		Ø % symptoms	Disease control
Check untreated uninfected	0		0		0		0	
Check infected	64	C	79	A	70	A	71	
Adepidyn 200 g/ha	1	B	19	C	4	B	8	89
Adepidyn 100 g/ha			35	B	10	B	23	68
Adepidyn 50 g/ha					3	B	3	96
Proline 200 g/ha	13	A	21	C	8	B	14	80
Proline 100 g/ha			33	B	12	B	23	68
Proline 50 g/ha					14	B	14	80
3DAA								
Check untreated uninfected	0		0		0		0	
Check infected	86	C	98	A	75	A	65	
Adepidyn 200 g/ha	3	B	29	CD	5	B	9	85
Adepidyn 100 g/ha			40	C	6	B	15	76
Adepidyn 50 g/ha					9	B	5	93
Proline 200 g/ha	17	A	23	D	18	B	14	78
Proline 100 g/ha			53	B	26	B	26	60
Proline 50 g/ha					31	B	15	76
5DAA								
Check untreated uninfected	0		0		0		0	
Check infected	100	C	78	A	55	A	78	
Adepidyn 200 g/ha	18	B	15	D	9	BC	14	82
Adepidyn 100 g/ha			29	CD	9	BC	19	76
Adepidyn 50 g/ha					2	C	2	97
Proline 200 g/ha	66	A	38	BC	25	B	43	45
Proline 100 g/ha			54	B	24	B	39	50
Proline 50 g/ha					8	BC	8	90
7DAA								
Check untreated uninfected	0		0		0		0	
Check infected	100	C	88	A	70	A	86	
Adepidyn 200 g/ha	12	B	15	C	0	C	9	89
Adepidyn 100 g/ha			16	C	8	BC	12	86
Adepidyn 50 g/ha					13	BC	13	84
Proline 200 g/ha	78	A	34	B	23	B	45	48
Proline 100 g/ha			77	A	21	B	49	43
Proline 50 g/ha					10	BC	10	89
9DAA								
Check untreated uninfected	1		0		0		0	
Check infected	100	C	99	A	94	A	98	
Adepidyn 200 g/ha	20	B	18	C	7	D	15	85
Adepidyn 100 g/ha			48	B	32	BD	40	59
Adepidyn 50 g/ha					16	CD	16	83
Proline 200 g/ha	52	A	53	B	42	BC	49	50
Proline 100 g/ha			92	A	29	CD	60	38
Proline 50 g/ha					57	B	57	42

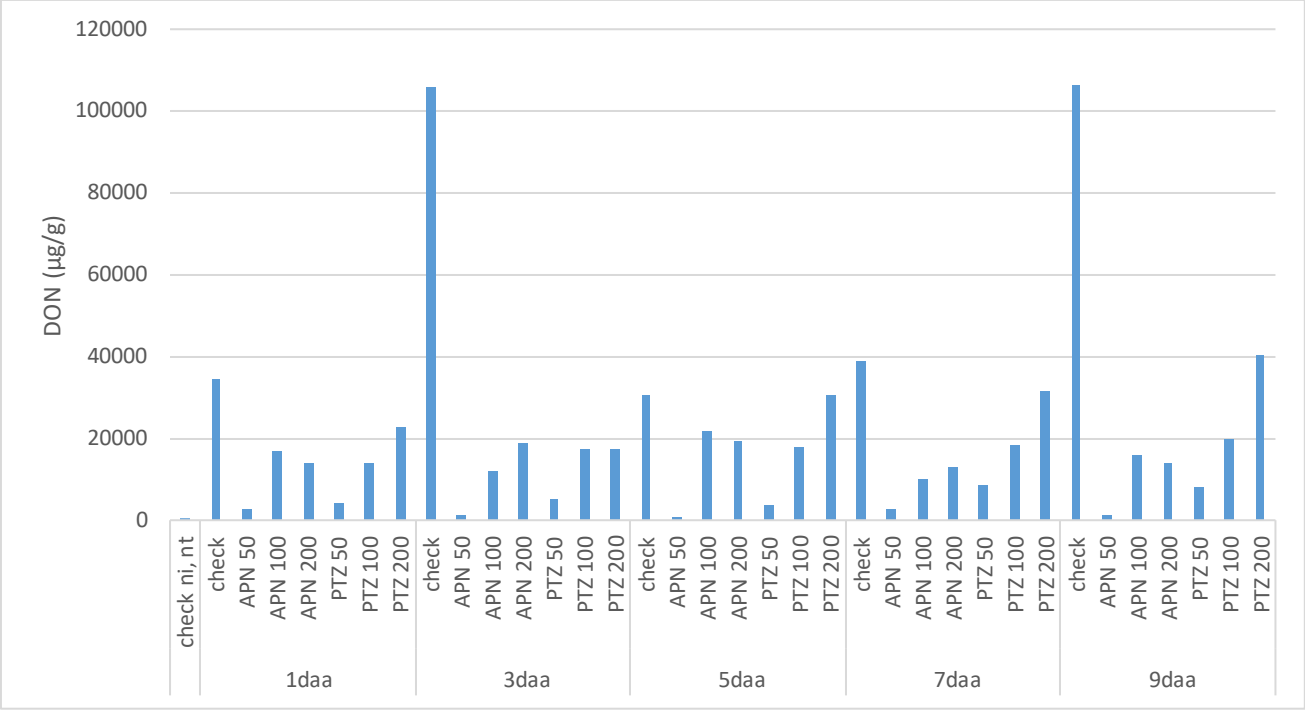

Figure 2: Mycotoxin amount produced by F. graminearum

4.3.2.2. Long preventive F. culmorum

All checks infected had good symptoms (between 17 and 100%- Table 5). At 1 daa on the rep-1 no statistics could be done but the trend shows a better efficacy on the symptoms of APN 200 g/ha and 100 g/ha (3 and 7%) compared to PTZ (16 and 21%). In rep-2 there was no significant differences between APN and PTZ 200 g/ha but DC was higher for APN (92%) than for PTZ (62%).

At 3 daa no significant differences was shown in rep-1 between the treatments but in rep-2 Proline 200 g/ha showed less symptoms (40%) than APN (67%). DC was significantly higher for PTZ 200 g/ha (77% to 60% for APN).

At 5 daa there was a significant difference in both reps with a better efficacy of APN 200 g/ha (7 and 6%) compared to PTZ 200 g/ha (22-39%). DC was better with APN, 93% than for PTZ 68%. At 7 daa in rep-1 there was a significant difference with a better efficacy with PTZ 200 g/ha (40% to 61% symptoms with APN) and in the rep-3 there was no significant difference but he trend of a better efficacy of APN 200 g/ha (19% to 26% symptoms with PTZ) is shown. DC was better with PTZ (57%) than with APN (65%).

Otherwise at 9 daa there was a significant difference between the treatments for both reps, with a better efficacy of APN 200 g/ha, 12% against 26% of symptoms with PTZ in rep-1 and 69% against

100% symptoms with PTZ in rep2. DC showed a higher efficacy with APN 200 g/ha (52%) than with PTZ (25%). The tendency showed a less good DC with a later infection (which means more time between the infection and treatment) and lower treatment doses showed a decreasing DC.

DON amounts decreased (Fig. 3) at 1, 5, 7 and 9 daa with APN treatments at 200 g/ha. At 1 daa DON amounts were at 1000 µg/g for APN against 10 000 µg/g for PTZ, at 5 daa DON amounts were at 50 000 µg/g for APN against 10 000 µg/g for PTZ, at 7 daa DON amounts were at 5000 µg/g for APN against 7000 µg/g for PTZ and at 9 daa DON amounts were at 18000 µg/g for APN against 40 000 µg/g for PTZ. In contrast at 3 daa DON decreased with PTZ 200 g/ha treatments (5000 µg/g against 10 000 µg/g with APN).

Table 5: Results of long preventive assay with F. culmorum*, Letters represent statistical differences between the treatments.*

Symptoms of the disease on the ears (%)

1DAA	Rep-1		Rep-2		Ø % symptoms	Disease control
Check untreated uninfected	0		0		0	
Check infected	31		17	A	24	
Adepidyn 200 g/ha	3		1	C	2	92
Adepidyn 100 g/ha	7		13	AB	10	58
Adepidyn 50 g/ha	17		8	BC	13	48
Proline 200 g/ha	16		2	C	9	62
Proline 100 g/ha	21		6	BC	13	44
Proline 50 g/ha	15		9	BC	12	49
3DAA						
Check untreated uninfected	0		0		0	
Check infected	94	A	100	A	97	
Adepidyn 200 g/ha	10	D	67	B	38	60
Adepidyn 100 g/ha	35	BC	71	AB	53	45
Adepidyn 50 g/ha	88	A	100	A	94	3
Proline 200 g/ha	12	CD	34	C	23	77
Proline 100 g/ha	41	B	52	BC	46	52
Proline 50 g/ha	76	A	100	A	88	9
5DAA						
Check untreated uninfected	0		0		0	
Check infected	87	A	100	A	93	
Adepidyn 200 g/ha	7	D	6	D	6	93
Adepidyn 100 g/ha	32	CD	20	D	26	72
Adepidyn 50 g/ha	30	CD	100	A	65	31
Proline 200 g/ha	39	BC	22	C	30	68
Proline 100 g/ha	73	A	46	B	59	37
Proline 50 g/ha	60	AB	62	B	61	34
7DAA						
Check untreated uninfected	0		0		0	
Check infected	88	A	100	A	94	
Adepidyn 200 g/ha	61	C	19	D	40	57
Adepidyn 100 g/ha	64	BC	44	C	54	43
Adepidyn 50 g/ha	77	AB	47	C	62	34
Proline 200 g/ha	40	D	26	D	33	65
Proline 100 g/ha	52	CD	72	B	62	34
Proline 50 g/ha	82	A	100	A	91	3
9DAA						
Check untreated uninfected	0		0		0	
Check infected	68	A	100	A	84	
Adepidyn 200 g/ha	12	C	69	B	40	52
Adepidyn 100 g/ha	10	C	100	A	55	35
Adepidyn 50 g/ha	48	AB	100	A	74	12
Proline 200 g/ha	26	BC	100	A	63	25
Proline 100 g/ha	35	BC	100	A	67	20
Proline 50 g/ha	62	A	100	A	81	4

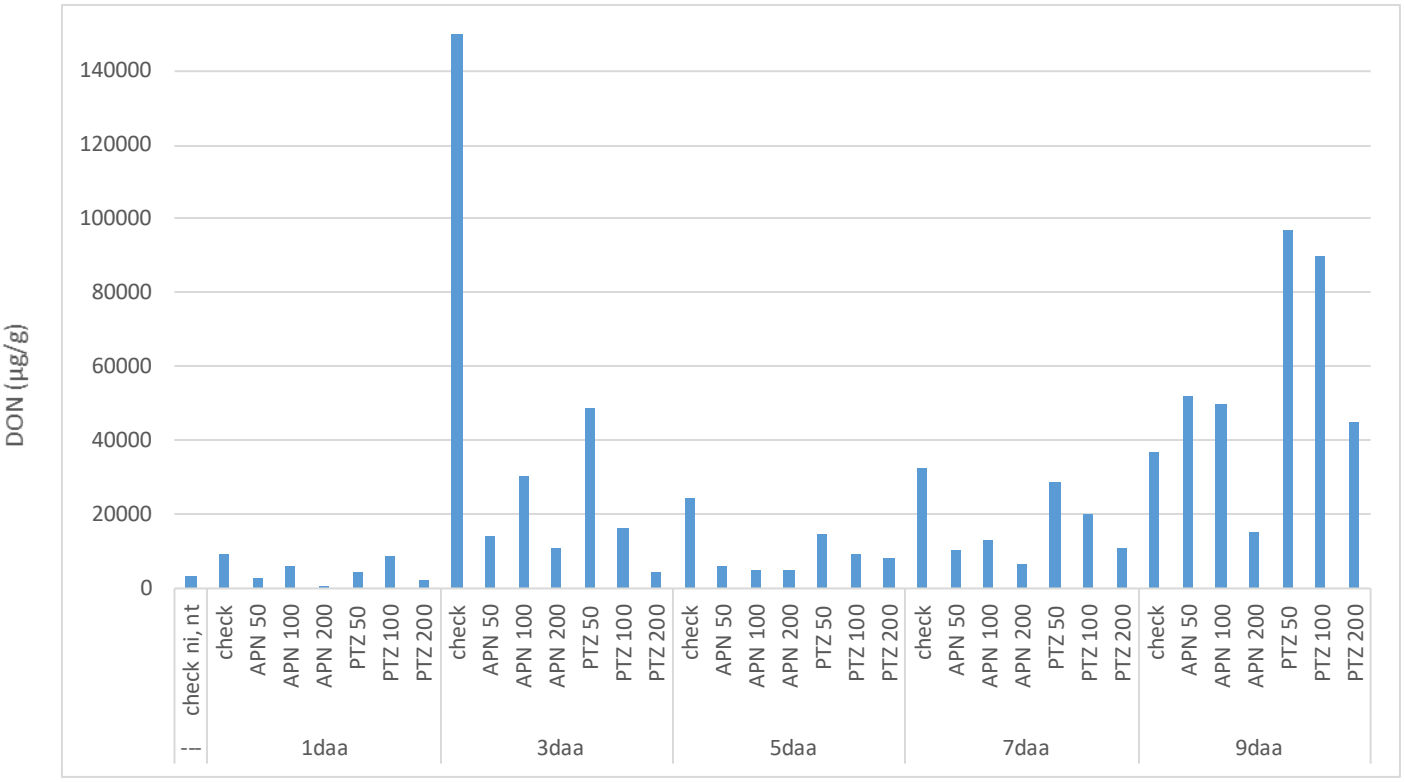

Figure 3: Mycotoxin amount produced by F. culmorum

4.3.2.3. Long preventive F. poae

Disease severity increased with a later inoculation (check infected at 1 daa had 16% symptoms and 75% at 9 daa, Table 6). At 1 daa no significant differences were shown between the treatments at higher rates (between 1 and 5% infection).

At 3 daa in rep-1 no significant difference was shown between the treatments but in rep-2 APN 200 g/ha showed a better efficacy (4% of symptoms against 11% with PTZ). DC was higher with APN 200 g/ha (88%) than for PTZ (71%).

At 5 daa and 7 daa there was again no significant differences between higher rates of fungicides. At 7 daa there was no significant differences between the treatments for rep-2 but for rep-1, APN 200 g/ha was significantly better (16%) compared to PTZ (24% of symptoms).

DON amounts (Fig. 4) were low at 1 daa and 3 daa even for the checks (less than 2500 µg/g), at 5 daa PTZ 200 g/ha decreased DON amounts compared to APN (5000 µg/ha against 13 000 µg/g) and the DON showed a high expression over this application timing. At 7 and 9 daa DON amounts decreased with both treatments (less than 2000 µg/g).

Table 6: Results of long preventive assay with F. poae, *Letters represent statistical differences between the treatments.*

	Symptoms of the disease on the ears (%)					
1DAA	Rep-1		Rep-2		Ø %symptoms	Disease control
Check untreated uninfected	0		0		0	
Check infected	16	A	17	A	17	
Adepidyn 200 g/ha	5	B	1	C	3	82
Adepidyn 100 g/ha	4	B	6	B	5	68
Adepidyn 50 g/ha	4	B	7	B	5	67
Proline 200 g/ha	0	B	3	C	1	92
Proline 100 g/ha	3	B	3	BC	3	83
Proline 50 g/ha	5	B	5	BC	5	71
3DAA						
Check untreated uninfected	0		0		0	
Check infected	15	A	32	A	23	
Adepidyn 200 g/ha	2	B	4	C	3	88
Adepidyn 100 g/ha	1	B	11	BC	6	73
Adepidyn 50 g/ha	4	B	8	BC	6	74
Proline 200 g/ha	3	B	11	BC	7	71
Proline 100 g/ha	3	B	8	BC	6	75
Proline 50 g/ha	3	B	18	B	11	54
5DAA						
Check untreated uninfected			0		0	
Check infected			55	A	55	
Adepidyn 200 g/ha			2	B	2	97
Adepidyn 100 g/ha			13	C	13	77
Adepidyn 50 g/ha			3	B	3	95
Proline 200 g/ha			5	B	5	91
Proline 100 g/ha			23	D	23	58
Proline 50 g/ha			17	D	17	69
7DAA						
Check untreated uninfected	0		0		0	
Check infected	75	A	66	A	70	
Adepidyn 200 g/ha	26	B	18	C	22	69
Adepidyn 100 g/ha	48	B	73	A	60	14
Adepidyn 50 g/ha	28	B	37	BC	32	54
Proline 200 g/ha	30	B	22	C	26	63
Proline 100 g/ha	44	B	35	BC	40	43
Proline 50 g/ha	48	B	60	AB	54	23
9DAA						
Check untreated uninfected	0		0		0	
Check infected	72	A	75	A	73	
Adepidyn 200 g/ha	16	C	17	C	17	77
Adepidyn 100 g/ha	39	BC	51	AB	45	39
Adepidyn 50 g/ha	47	B	33	BC	40	46
Proline 200 g/ha	24	BC	15	C	19	74
Proline 100 g/ha	40	BC	38	BC	39	47
Proline 50 g/ha	33	BC	73	A	53	28

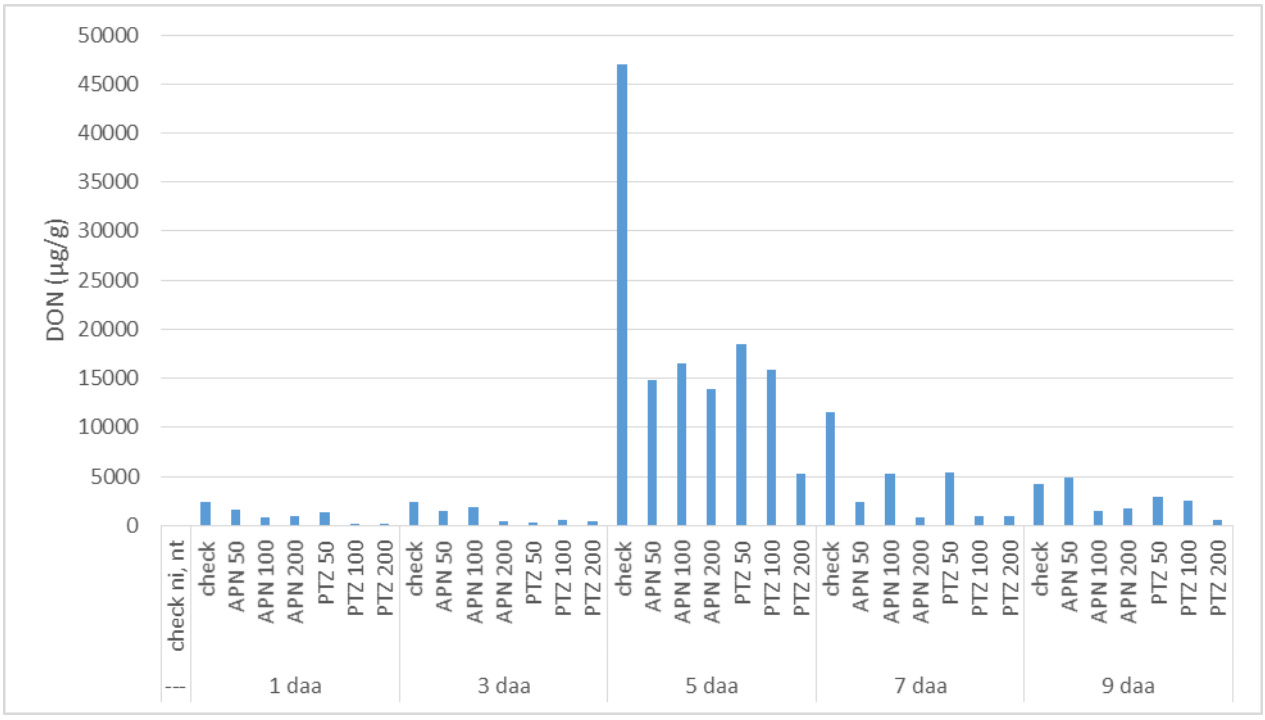

Figure 4: Mycotoxin amount produced by F. poae

4.4. Discussion

Reduction of FHB and mycotoxin contamination in wheat were reported with contrasting results so far: whereas studies showed a control of disease and DON accumulation with an application during later development stages of wheat [69] others showed a decrease of FHB with early applications [73]. It is a crucial issue as *Fusarium* infected plants during anthesis [29] and mycotoxins are produced during the first stages of infection [33]. In this study it was shown that Fusarium species are able to infect wheat even after 12 days of flowering. This suggest that application timing should not only be focused on anthesis.

Long curative application timings showed that even treating plants after 12 days of inoculation, disease control with adepidyn (APN) was still very promising. DON levels increased after 10 days of inoculation but decreased again after 12 days. Disease intensity was related to toxin concentration which is not in correlation with other studies done [105]. Long preventive assays were performed on *F. graminearum*, *F. culmorum* and *F. poae* as they are all part of the most frequently encountered species [23] and produce DON mycotoxins. Plants were all treated during flowering of wheat and inoculated at different time points after treatments. Results showed a very good efficacy of APN for all three pathogen systems with an increase of disease control even if inoculated 9 days after application. DON concentrations of *F. graminearum* did not increase even with an infection 9 daa. DON concentrations produced by *F. culmorum* did increase very slowly after 9 daa and DON amounts produced by *F. poae* were generally very low, however DON concentrations were very high at 5 daa. This time point should be considered in further assays done with *F. poae*. Some results showed a higher DC control and less mycotoxin amounts for lower fungicide rates. It is known that sub-lethal doses of fungicides can induce stress signaling pathways by fungal pathogens and they are able to resist against chemical attacks [106].

Plants were grown in the greenhouse and assays were spread out during several months. Even if climatic conditions can be controlled, external light intensity plays a crucial role in plant development with a faster development in summer. Growing period of plants might play a role in plant fitness and can lead to some differences between assays performed identically.

It was shown in this chapter that application timing with adepidyn to control *Fusarium* species and their mycotoxin production is flexible. However, these conclusions should be tested in the field under real conditions.

5. FHB control and mycotoxin management under field conditions with a novel fungicide

5.1. Introduction

Cereals are among the key crops in agriculture, with an estimated production of 3.75 billion tons per year worldwide [2]. Cereal production includes wheat, rice, oat, barley and corn, and wheat is the staple food for the majority of the world's population [107]. Climatic conditions with warm and wet weather promote the development of fungal diseases on wheat such as the Fusarium head blight complex (FHB) and the production of mycotoxins in the germ associated with this disease. Thirteen *Fusarium* species have been reported causing damage on wheat and corn [24, 93]. Some of these species are specific to a particular crop and others are able to infect diverse crops, which has been shown to increase mycotoxin contents in the grains [52]. In the past, FHB caused severe damages with disease incidence between 20 and 100%, and losses were estimated at several million US dollars [108]. These losses include trichothecene contamination for example by deoxynivalenol (DON), which is the predominant mycotoxin found in Europe [22, 50]. The mycotoxins cause a wide range of chronic and severe effects in human and animals and lead to multiple diseases including immunosuppression, alteration in growth and development, teratogenicity and cancer [49, 50]. Mycotoxins also decrease the quality of the harvest, which cannot be used at all by the food industry if threshold concentrations are exceeded. There are legislative limits for DON, zearalenone and fumonisins [109] and reducing their amount is not only important for human and animals health but also for farmers as contaminated grains cannot be sold.

So far, there is no single control method available for FHB and mycotoxin production. However, a combination of methods can be applied to reduce the risk. At first is appropriate cultivation practice. It was shown that minimizing fertilization in conventional agriculture reduces *Fusarium* and toxin amounts [110]. Other ways of controlling FHB include their control with bio-control agents [111], genetic control and chemical control [28]. In the past, fungicides from the class quinone outside inhibitor (QoI) and demethylation inhibitor (DMI) were already tested but did not lead to a satisfactory reduction of DON contamination [72]. Prothioconazole, namely proline, a DMI fungicide, showed to be the most efficient against *Fusarium* species [70]. Recently, Syngenta developed a new fungicide, adepidyn (APN; active ingredient is pydiflumetofen), which is part of a new generation of succinate dehydrogenase inhibitor (SDHI) fungicides [84] with high intrinsic activity. SDHIs bind strongly to the ubiquinone-binding site in the mitochondrial respiratory chain of fungal pathogens called complex II

or SDH enzyme [80]. Currently the overall spectrum of SDHI fungicides is broad and different chemical structures of them exist, which is of high interest to prevent resistance [83].

The aim of this work was to test the efficacy of the new fungicide adepidyn under field conditions on *Fusarium* species and the effect on mycotoxin production. Experiments were conducted on wheat and corn to investigate the *in-planta* activity of this fungicide and in order to determine the efficacy of the new fungicide under realistic conditions, compared to proline (prothioconazole; DMI; PTZ) and caramaba (metconazole; QoI). We also experimented on the timing of application to assess curative and preventive efficacy of adepidyn under field conditions. These studies give insights into putative differential behavior of *Fusarium* species under chemical control in field situations.

5.2. Material and Method

5.2.1 Isolates used for inoculations

For the wheat assay, a liquid mixture of three *F. graminearum* isolates was prepared at a concentration of 200 000 spores/ml. The isolates used were K6139 (CS-FU00123), K6934 (CS-FU00124) and K6935 (CS-FU00125) (description in Table 1, Appendix). They were selected based on their good sporulation, visible symptoms and mycotoxin production. Isolate were first grown on plates with PDA medium (potato dextrose agar, 39 g/L) for 12 days at 65% relative humidity (RH), 12 hours dark and 12 hours UV light, 21°C. An *in vitro* assay was performed to select these isolates (chapter 2) for them to be in a median sensitivity to APN.

For the corn assay, two *Fusarium* treatments were used, consisting of different species. In a first round of the assay, the same mixture of *F. graminearum* was used as for the wheat assay. Furthermore, an *F. verticillioides* isolate was used. Also, the latter isolate was grown on plates with PDA medium for 12 days at 65% RH, 12 hours dark and 12 hours UV light. Then wheat seeds were inoculated with both pathogens for 12 days. After drying the seeds, they were ground to get a powder used for the inoculation.

5.2.2. Field assay of curative and preventive application on wheat

5.2.2.1. Wheat assay

Curative assay in 2017 was done on winter wheat (variety Tapidor): the experiment took place in Waldacker 1, Stein, Switzerland (latitude: 47.55° N; longitude: 7.97° E; altitude: 302m). The field assay

was composed of 15 plots (3.2 m x 4.5 m) and 500 seeds per m^2 were sown on 20.10.2016. Per plot 50 ears were first marked with a cable tie and then one spikelet per ear was marked with a permanent marker. Ears were infected (1 ear per plant) with the *F. graminearum* spore suspension by pipetting 10 µl in each marked ear. Four days later, five treatments (Table 1) with three replicates per treatment (3 plots) were randomly assigned to the 15 plots and applied on 29.05.2017. Treatments included check not inoculated-not treated, check inoculated, adepidyn EC (emulsifiable concentrate), proline and caramba. Fungicide application of each plot was performed at BBCH 61-63 by a tractor mounted with a Boom Sprayer, for cereal T3 treatment using angled flat fan nozzles FLDOOU Altbuz Twin AVI1101 (Hedinova, Syngenta) at 3 bars. Humidification of the plots was done regularly the days after the inoculation (in the morning and in the evening for 1 hour). After 14, 21 and 28 days after inoculation, ears were assessed for the percentage of infection visible on each ear. At complete maturity, ears were harvested, dried and cleaned for assessing the thousand grain weight (TGW) and for mycotoxin analysis (done by Qualtech group, Nancy, France).

Preventive and curative assays were performed in 2018 on winter wheat (Tapidor). The experiment took place in Waldacker 2, Stein, Switzerland (latitude: 47.80° N; longitude: 5.95° E; altitude: 297m). The field assay consisted of 24 plots (3.2 x 4.5m) and 500 seeds per m^2 were sown on 19.10.2017. Curative and preventive fungicide treatments were applied to the same plots, and application of fungicide happened at the same time, but the timing of inoculation differed (Table 1). At the beginning of flowering, 2x 50 ears per plots (1 ear per plant e.g., 50 plants) were marked with a cable tie (yellow for the curative assay and red for the preventive assay) and a single spikelet on these ears was marked with a permanent marker. The ears marked with the yellow cable tie were first inoculated (21.05.2018) with the *F. graminearum* spore suspension by pipetting 10 µl in each marked ear. Fungicide application of each plot was performed 4 days later at BBCH 61-63 by a tractor mounted with a Boom Sprayer, for cereal T3 treatment using angled flat fan nozzles FLDOOU Altbuz Twin AVI1101 (Hedinova, Syngenta) at 3 bars. Treatments were check not inoculated-not treated, check inoculated, adepidyn EC, adepidyn SC (suspension concentrate), proline and caramba, with 4 replicate plots per treatment. The second inoculation (preventive fungicide treatment) took place 3 days after fungicide application, and again the ears marked with the red cable tie were inoculated (28.05.2018) with the *F. graminearum* spore suspension by pipetting 10 µl in each marked ear. After each inoculation, plants were irrigated and the humidification was done regularly twice in a day. After 14, 21 and 28 days after inoculation, ears were assessed for the percentage of infection visible on each ear. At complete maturity, ears were harvested, dried and cleaned for assessing the thousand grain weight (TGW) and for mycotoxin analysis (done by Qualtech group, Nancy, France).

The preventive and curative assays in 2019 were done the same way as in 2018. However, winter wheat Baretta was used which was sown on 24.10.2018. Treatments (table 1) were performed on 07.06.2019, 2 days after the first inoculation and 3 days before the second. Assessment were performed at 14 days and 21 days after the inoculations. Plants were treated with different maintenance products during growth and crop development (Appendix Table 2).

Table 1: Adepidyn (pydiflumetofen), Switzerland, foliar use, SDHI, Syngenta; Prothioconazole=proline, Germany, systemic, DMI, Bayer; Metconazole=Caramba, Germany, systemic, DMI, BASF

Date of assay and application	**2017**	**2018**	**2019**
Wheat variety	Tapidor	Tapidor	Baretta
Treatments /no. of plots			
Check untreated, unincoculated	3	4	4
Check inoculated	3	4	4
Adepidyn EC 62.5,	3	4	4
Adepidyn SC200 + Agral	0	4	4
Prothioconazole Proline EC 250	3	4	4
Metconazole Caramba Star SL90	3	4	4

5.2.3. Field assay on corn

In the years 2017 and 2018, two parallel corn assays were performed. The first was a variety assay to choose new hybrids for the upcoming year and the second was a fungicide treatment corn assay. in year 2019, a fungicide treatment corn assay was performed.

5.2.3.1. Variety assay

In 2017 the variety assay experiment was designed with 6 rows of different hybrids listed in Table 2 (each row had a different hybrid), plants were spaced by 13.5 cm to each other and space between the rows was 75 cm. Each row had a length of 200 m and two external buffer rows were sown (both rows were not used for the assay). Each row was split in two (2x 100m). The first part of the row was again split in 10 equal plots and was used to test a spraying method which consisted on putting a spore suspension in a spray bottle, which was then used to inoculate the silks of the plants (silks 3 cm length). Spore suspension used are listed in Table 3. The second part of the row was split in 11 equal plots and was used to test a wounding assay, using forks which were put in a spore suspension and then put in the ear of each plant of the plot. The assessment was done at full maturity. The quality of the ear was estimated by estimating the percentage of damage (damaged ears meaned ear with no seeds, ears

eaten by the birds). Percentage of infection of each pathogen on each hybrid was also estimated and grains were collected for toxin analysis.

In 2018 8 hybrids (Table 2) were tested. Again 8 rows of 200 m and two external buffer rows were sown with 13.5 cm between the plants and 75 cm between each row. Border rows not used were also filled with seeds. Two inoculation methods were tested and each row was divided into two parts. In the first part of the row of each hybrid a *F. graminearum* mixture (K6934 = CS-FU00123, K6935 CS-FU00124, K6139 = CS-FU00125) was inoculated using a syringe at full silking by putting 1 mL of spore suspension under the beginning of the silks on the ears. The second part of the plots were divided into three plots and three *F. verticillioides* isolates were tested (CS-FU00135=135, CS-FU00449=449, CS-FU00460=460) by using forks and a powder of dried wheat kernels infected by the isolates. Forks were put into 20 cm long ears (two weeks later than the *F. graminearum* inoculation). The assessment was done at full maturity. The quality of the ear was estimated by estimating the percentage of damage (ear with no seeds, ears eaten by the birds). Percentage of infection of each pathogen on each hybrid was also estimated and grains were collected for toxin analysis.

Table 2: Hybrids used for the corn variety assay

Hybrid	Origin	Year
ES Metronom	Euralis Saaten GmbH	2017
Avenir	France	2017, 2018
Kroissan	KWS	2017
Adevey	Advantin/Limagrain Europe	2017
P8400	Pioneer	2017
Figaro	KWS	2017
Farmicus	FarmSaat AG	2018
SA0025	Syngenta	2018
Talisman	Syngenta	2018
Crossman	Euralis Saaten GmbH	2018
Farminion	Samen STEFFEN AG	2018
Ricardinio	KWS	2018
Laurinio	KWS	2018

Table 3: Different spore suspensions used for the variety assay 2017 and two inoculation methods. Each spore suspension was tested on each hybrid.

Spray assay	Wounding assay
Spore suspension	Spore suspension
F.verticilliodes 1milion sp/mL	*F.verticillioides* K6335
F.verticillioides 100' 000 sp/mL	*F.verticillioides* K6334
M. nivale 100'000 sp/mL	*F.verticillioides* K6333
M. majus 100'000 sp/mL	*F.verticillioides* K6332
F. avenaceum 100'000 sp/mL	*F.verticillioides* K6331
F. tricinctum 100'000 sp/mL	*F.verticillioides* K6330
F. culmorum 100'000 sp/mL	*F.verticillioides* K6155
F.graminearum K6934 100'000 sp/mL	*F.verticillioides* K6147
F.graminearum K6935 100'000 sp/mL	*F.verticillioides* K6146
F.graminearum K6139 100'000 sp/mL	*F.verticillioides* K6145
Check not infected	*F.graminearum* K6935, K6934, K6139
	Check not infected

5.2.3.2. Assessment of the ears

Ears were assessed by the percentage of symptoms visible on the ears (Fig. 1).

1- 0%, no infection
2- 4-10%
3- 11-20%
4- 20-50%
5- 51-100%

Figure 1: Assessment of F. graminearum *(left) and* F. verticillioides *(right). (V. Ortega)*

5.2.3.3. Fungicide treatments on corn

A preventive corn assay was performed in 2017, 2018 and 2019. In the first year, ears were inoculated with a mixture of *F. graminearum*, and in the following two years, ears were inoculated with the same mixture of *F. graminearum* and with *F. verticillioides*. Plants were treated with different maintenance products from April to June (Appendix Table 3).

- ***2017***

ES Metronom grains were sown on 05.05.2017 (Strassenacker 1, Stein, Switzerland; latitude: 45.82° N; longitude: 10.46° E, altitude: 301m). Design of the corn assay was composed of 40 plots, divided in four groups (Fig. 2). The first group was four plots with no treatment and no inoculation. The three other groups all contained four plots with the following treatments: none but inoculated (check), adepidyn (EC62.5, A21857B, 200 g/ha, 3.2 L/ha, Syngenta, Switzerland), and prothioconazole (proline, EC250, EXC12, 200 g/ha, 0.8 L/ha, Bayer, Germany). Each group was treated with different application methods and timings:

1. V8-V10, flat fan, 150 L/ha (22.06.2017) with a Boom Sprayer 3.0 Bar, Nozzle FLDOOU Altbuz AVI11001T Hedinovo Bothpth Syngenta
2. R1 (silking) flat fan, BBCH 61-63, 150 L/ha, (or 300L/ha), 13.07.2017) with a Boom Sprayer 3.0 Bar, Nozzle FLDOOU Altbuz AVI11001T, Hedinovo Bothpth Syngenta
3. R1: New technology facilitating better treatment of ears (13.07.2017), Boom Sprayer 3.5 Bar, Nozzle Defy 04, Hedinovo Bothpth Syngenta

Each plot had four plant rows. Both external rows were buffer rows and both inner plots were the plots inoculated. Twenty ears per row (40 ears per plot) were inoculated at full silking (200 000sp/mL) on 14.07.2017 by using a spray bottle and spraying on the ears. Assessment of the ears was done at full maturity of the ears, when harvest conditions were optimum by estimating the percentage of infection. Ears were dried 14 days in the greenhouse and seeds were threshed for toxin analysis focusing on DON and Fumonisin, done by Qualtech group (Nancy, France) .

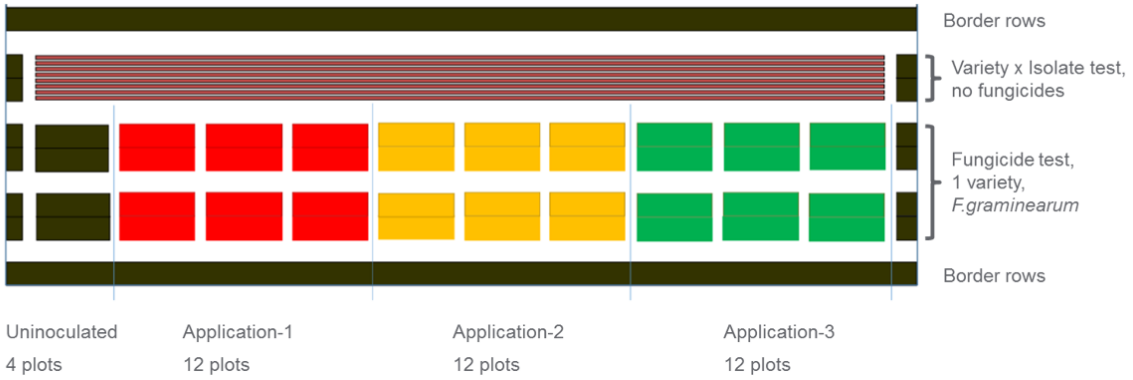

Figure 2: Design of the corn assay plots 2017. Red plots are those treated with the application method 1, yellow with the application method 2 and red with the application method 3. Black spots are border or buffer plots.

- ***2018***

Avenir seeds were sown on 08.05.2018 (Strassenacker 1, Stein, Switzerland; latititude: 45.82° N; longitude: 10.46° E; altitude: 301m). Plot design was composed of 16 plots (Fig. 3A and B) and each plot contained four rows of plants (18.5 cm, 13.5 cm between each plant and 75 cm between the rows). Both border rows were buffer rows and both inner plots were either inoculated with F. *graminearum* or with F. *verticillioides* (figure). Forty ears per row were inoculated. Four treatments were done in this assay. Check untreated-uninoculated, check inoculated, adepidyn (EC62.5, A21857B, 200 g/ha, 3.2 L/ha) and prothioconazole (Proline, EC250, EXC12, 200 g/ha, 0.8 L/ha). Application was done at R1, BBCH61-63 at full silking and with a Boom sprayer – tractor mounted-horizontal, Nozzle type: IDKT120-02 at 5 bars. Application was performed on 07.07.2018.

F. graminearum mixture (K6139, K6934, K6935 at 200 000sp/ml (1:1:1 mixture)) inoculation was done on 09.07.2018, two days after the applications by using a syringe through the spathes and the volume injected was 1 mL (Fig.3C). *Fusarium verticillioides* K6155= CS-FU00135 was inoculated on 16.07.2018, 9 days after the application (full developed ear, 20 cm and 14 days after flowering) by using a powder (grinded wheat seeds infected with the pathogen). A fork was put into water, then into the powder and pushed into the ear. Humidification was done for 2 hours after each inoculation and every day in the morning and the evening for 44 days after the infection. Assessment of the ears was done as in 2017.

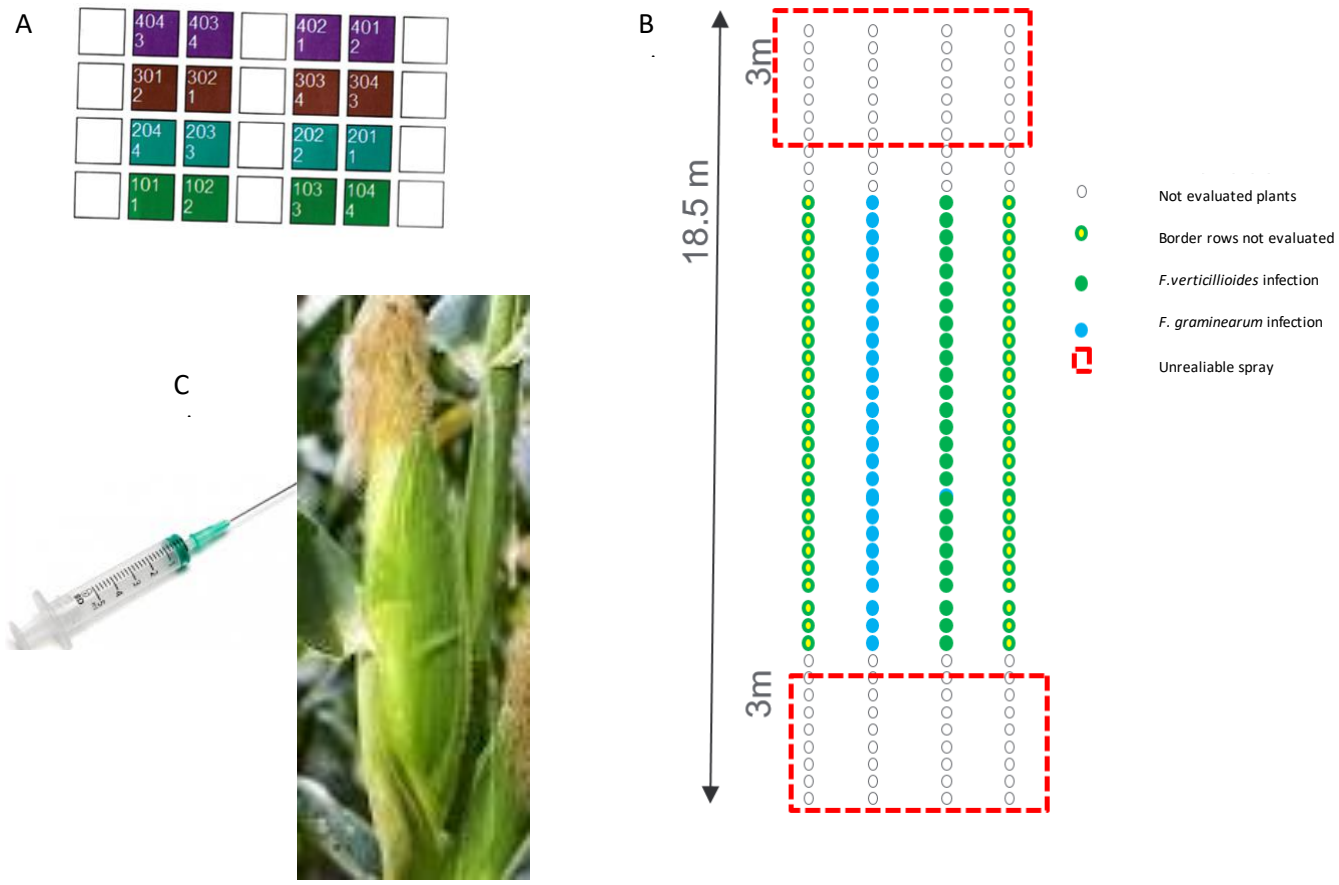

Figure 3: A. design of field assay, colors correspond to the different replicates and numbers to the treatments. 1. Check ni, nt, 2. check I, 3. APN, 4. PTZ. B. Plot design with border rows and blue F. graminearum *inoculation, green* F. verticillioides *inoculation. C. Inocuation into the ears*

- ***2019***

Variety Talisman was sown on 24.04.2019 (Strassenacker 1, Stein, Switzerland; latitude: 45.82° N; longitude: 10.46° E; altitude: 301m) and 24 plots were created (Fig. 4A). Each plot was also containing four rows of plants (18.5 cm, 13.5 cm between each plant and 75 cm between the rows). Both border rows were buffer rows and both inner plots were either inoculated with *F. graminearum* or with *F. verticillioides* (Fig. 3B). Forty ears per rows were inoculated. Six treatments were done in 2019.

1. Check not inoculated, untreated (1)
2. Check inoculated (2)
3. Adepidyn (APN) at R1 (3) , A21857, 62.5 GA/L, 200gai/ha, 3.2L/ha
4. Adepidyn (APN) at V8-V10 (4) , A21857, 62.5 GA/L, 200gai/ha, 3.2L/ha
5. Proline at V8-V1 (PTZ) 0 (5), Prothioconazole, 250EC, 250GA/L, 200 gai/ha, 0.8 L/ha
6. Proline at R1 (6 , (PTZ) Prothioconazole, 250EC, 250GA/L, 200 gai/ha, 0.8 L/ha

Fungicide application of treatments 4 and 5 were done on 10.07.2019 (V8-V10) and applications of treatments 3 and 6 on 19.07.2019 (R1) with a Boom Sprayer, FLDOOU, 5 Bar (Syngenta).

F. graminearum mixture (K6139, K6934, K6935 at 200 000sp/ml (1:1:1 mixture)) inoculation was done on 22.07.2019, by using a syringe through the spathes and the volume injected was 1 mL (Fig. 3C). *F.verticillioides* K6155= CS-FU00135 was inoculated on 29.07.2019, (full developed ear, 20 cm and 14 days after flowering) by using a powder (grinded wheat seeds infected with the pathogen). A fork was put into water, then into the powder and pushed into the ear. Humidification was done for 2 hours after each inoculation and every day in the morning and the evening for 47 days after the infection. Assessment of the ears was done at full maturity of the ears, when harvest conditions were optimum by estimating the percentage of infection. Seeds were threshed at the mean time by using a thresh machine (Fig. 4B) and seeds were analyzed for toxin by Qualtech group (Nancy, France), for DON and Fumonisin. During the entire phase of the experiments, plants were treated with different maintenance products (Appendix Table 3).

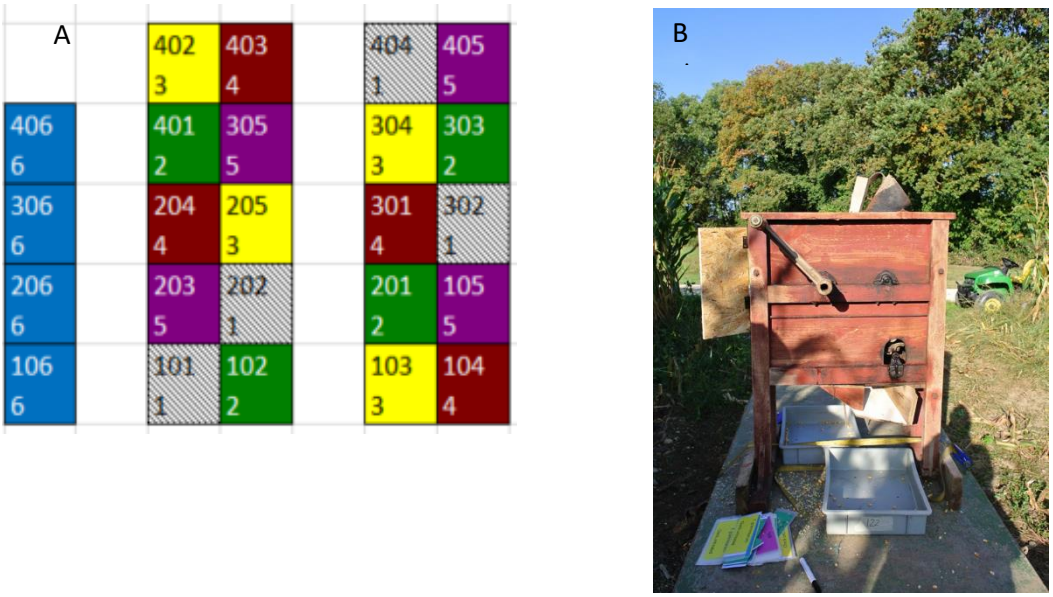

Figure 4: A. Design of the plots. Colors are replicates and numbers the diferent treatments. Grey check ni, nt, green check I, yellow APN V8-10, red APN 1, pink PTZ V8-10, blue PTZ R1. B. Thresh machine manual.

5.2.4. Statistical analysis

Data from the inoculated plots were subjected to analysis of variance using the Syngenta in-house package Acsapwin. The terms in the statistical model were treatment and block. Prior to analysis, percentages were arcsin-transformed, i.e. y=arcsin√(x/100), so as to better meet the assumptions upon which the validity of the analysis depends. The statistical significance of the overall effect of treatment was assessed via an F-test. In cases where the F-test was significant at the 5% probability level (i.e. F-test probability<5%), the significance of differences between specific treatments, including the inoculated check, was assessed using the LSD (least significant difference) method. Means on the transformed scale that differed by more than the relevant LSD were considered significantly different at the 5% probability level, providing evidence of a genuine difference between the two treatments in question. Differences that were smaller than the relevant LSD were interpreted as no greater than we would expect to see simply because of random variation, and did not, therefore, provide convincing evidence of a genuine difference between the two treatments in question. The outcome of all possible treatment comparisons was summarized in the form of a letter test such that means with no letter in common are significantly different.

5.3. Results

5.3.1. Management of *F. graminearum* and DON in wheat

Ears were assessed for disease by the percentage of symptoms visible. In all three years of assessment, in 2017 to 2019, the curative assay showed that the infection worked well between 30 and 52% of infection (Table 4). The external infection on uninoculated plots was low between 0 and 7%. Again, in three years the trend was the same with a better efficacy of APN EC (7-12% infection), proline (13-21%) and caramba (17-34%). In the three replicates caramba showed less good efficacy even compared to proline, 4-13% lower and the differences were significate. The efficacy of APN SC is not different than the efficacy of proline in 2018 and 2019 between 12 and 27% for APN SC and 13-21% for proline. Analysis of disease control on all three years together showed a better efficacy of APN EC 73.1%. The trend gives a DC of 51.2% for APN SC and 61 % for proline and caramba 45.4%. Proline (61%) is better than APN SC (51.2%) but not better than APN EC (73.1%).

In all three years, TGW was increased (1.5 g to 6.5 g) in samples treated compared to the check inoculated, sometimes even compared to the check untreated in 2018 (1.5 g) and 2019 (2.5 g) (Table 5). Ears treated with APN EC and APN SC had heavier grains (4.9 g and 4.6 g) than those treated with proline (3.6 g) and caramba (1.1 g). Mycotoxin analysis showed a decrease in DON contamination in grains treated with APN EC (1191 µg/g) compared to other treatments. Proline showed a bigger decrease in DON amount than APN SC (2082 µg/g to 2437 µg/g). Grains treated with caramba had the most DON amount (3258 µg/g).

Table 4: Curative assay wheat 2017, 2018, 2019. Information on statistically supported differences (different letters) is given.

	Fraction of surface with disease(%)						*Disease control*				
	2017	Letter	2018	Letter	2019	Letter	2017	2018	2019	DC	Letter
Check untreated, uninoculated	0		7		4						
Check inoculated	52	A	30	A	47	A	0.0	0.0	0.0	0	A
Adepidyn EC 62.5	12	C	7	C	16	C	76.9	76.7	66.0	73.1	C
Adepidyn SC200 + Agral	…		12	BC	27	BC	…	60.0	42.6	51.2	BC
Prothioconazole Proline EC 250	15	BC	13	BC	21	BC	71.2	56.7	55.3	61.0	BC
Metconazole Caramba Star SL90	18	B	17	B	34	AB	65.4	43.3	27.7	45.4	B

Table 5: toxin analysis (µg/g), thousand grain weight (TGW) and fungal biomass (µg/g) of the curative wheat assays in all three years

	2017			2018			2019					
Treatments	Toxins DON (µg/g)	TGW (g)	Fungal biomass	Toxins DON (µg/g)	TGW (g)	Fungal biomass	Toxins DON (µg/g)	TGW (g)	Fungal biomass	Toxins DON (µg/g)	TGW (g)	Fungal biomass
Check untreated, uninoculated	200	45	1	6000	45	0	225	40.5		2141	43.5	
Check inoculated	4100	39.5	1700	2800	41	7200	75	38.9		2325	39.8	
Adepidyn EC 62.5	1600	43.9	650	1900	47.5	1500	75	42.7		1191	44.7	
Adepidyn SC200 + Agral	…	…	…	4800	47.8	1600	75	41.1		2437	44.4	
Prothioconazole Proline EC 250	1800	43	1100	4200	45	2100	248.5	42.3		2082	43.4	
Metconazole Caramba Star SL90	1900	41	1200	7800	42.5	3000	75	39.2		3258	40.9	

Preventive assay was performed in 2018 and 2019. Reported in the Table 6 are assessments 14 dai for the year 2018 and 21 dai for the year 2019 (most relevant assessments because of weather differences). For both years there are no significant differences between a preventive application with APN EC (5% to 7%) and Proline (14% to 13%). Caramba has a very low efficacy with a preventive application (3.26% of disease control). TGW analysis (Table 7) showed heavier grains on treated (42.5 g to 45 g) ears and grains treated with APN EC showed heavier grains (45 g) than those treated with Proline (43.8 g). Mycotoxin analysis were done in 2019 and showed a decrease of DON amount for treated ears with APN EC (75 µg/g), APN SC (75 µg/g) and metconazole (75 µg/g). Prothioconazole showed also a decrease of DON compared to the check inoculated (150 µg/g) but less than the other fungicides.

Table 6: Preventive assay wheat 2018, 2019. Information on statistically supported differences (different letters) is given.

Preventive assay field wheat

	Disease area (%)				Disease control			
	2018	Letter	2019	Letter	2018	2019	DC	Letter
Check untreated, uninoculated	3		1		100.0	100.0	100	
Check inoculated	12	A	46	A	0.0	0.0	0	A
Adepidyn EC 62.5	5	B	14	B	58.3	69.6	63.94928	B
Adepidyn SC200 + Agral	6	AB	12	B	50.0	73.9	61.95652	B
Prothioconazole Proline EC 250	7	B	13	B	41.7	71.7	56.7029	B
Metconazole Caramba Star SL90	12	A	43	A	0.0	6.5	3.26087	A

Table 7: toxin analysis (µg/g), Thousand grain weight (TGW) and fungal biomass (µg/g) of the preventive wheat assays in 2018, 2019

	2018		*2019*		*Average*
	TGW (g)	Toxins DON (µg/g)	TGW (g)	Toxins DON (µg/g)	TGW (g)
Check untreated, uninoculated	45.8	225	39.1	225	42.45
Check inoculated	40.5	225	37.9	225	39.2
Adepidyn EC 62.5	47.5	75	42.5	75	45
Adepidyn SC200 + Agral	47.8	75	37.9	75	42.85
Prothioconazole Proline EC 250	46.2	150	41.5	150	43.85
Metconazole Caramba Star SL90	45.8	75	39.2	75	42.5

5.3.2. Management of *F. graminearum*, *F. verticillioides* and their toxins in corn

5.3.2.1 Hybrid selection

The aim of the variety assays was to select best fungal pathogens for the inoculations of the following years and to select the best corn hybrid adapted to Stein soils and having the best developed and fulling ears. Another aim was to confirm if the inoculation methods of the different pathogens can be used in following years. In 2017, ES Metronom had the worst ear damage, Avenir, Adevey and Figaro had the most homogenate ears and Kroissan and P8400 had too heterogeneous ears. The pathogen infections for the wounding assay showed a good infection of all isolates on all hybrids and the F. *graminearum* mixture showed the best infection on Avenir. *F. verticillioides* K6145 isolate produced most symptoms and fumonisin toxins. Using the spray inoculation method, *F. graminearum* showed most symptoms on Avenir. The infection impact on other variety with *F. graminearum* was too low with this method. P8400 or Kroissans had the second-best sensitivity to this pathogen by wounding, but the ear quality was not the best. Avenir seemed to be the most sensitive hybrid of *Fusarium* and was used for 2018 corn assay. In 2018 *F.* graminearum mixture showed good infection on all hybrids although the most severe symptoms were noticed on Laurinio and Avenir and Crossman. Farmicus, Talisman and Crossman were the most sensitive hybrids to *F. verticillioides* and isolates CS-FU00135 (K6155) and CS-FU00449 had most symptoms. Laurinio, Crossmann and Talismann had the highest DON amount and Farmicus, Talisman and Crossman the highest fumonisin amounts.

5.3.2.2 Pest and toxin management on corn

- 2017

The year 2017 was not successful for the corn assay, because of the very bad ear quality of ES Metronom. Ears were too much damaged and infection did not occur (Fig 5A). Years 2018 and 2019 were more successful for the corn assay. Infections of both *F. graminearum* and *F. verticillioides* worked well (Fig.5B, C, D, E). The assay could therefore be assessed.

Figure 5: A. 2017, bad ear quality ES Metronom. B. 2018 F. graminearum. *C. 2018* F. verticillioides. *D. 2018* F. graminearum. *E. 2019* F. verticillioides

- **Assay 2018**

Infection was good for *F. graminearum* (30%, Fig.6) and *F. verticillioides* (20%, Fig. 7). However, *F. graminearum* infection could statistically not be differentiated between check inoculated and treated plots although in rep 4 treated ears with adepidyn and proline showed a low decrease of the symptoms (10%). Ears infected with *F. verticillioides* and treated with adepidyn or proline showed statistically a decrease in symptoms but both treatments could not be differentiated (both 12% infection).

Toxin analysis (Table 8) showed high level of DON production for *F. graminearum* in treated (16000 ppb) and untreated (14000 ppb) ears. The same showed fumonisin analysis for *F. verticillioides* with values reaching 80000 ppb for treated and untreated ears. Fungal biomass assays showed the presence of the pathogens in the ears and confirmed the strong symptoms visible in the treated plots.

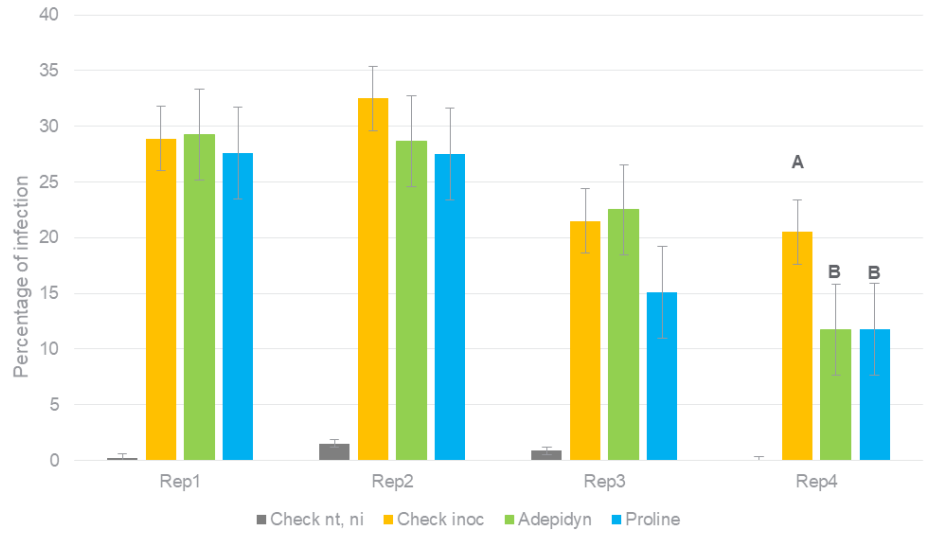

Figure 6: Percentage of infection of F. graminearum *in the different replication plots and for the four treatments.Means with standard deviation are presented, together with information on statistically supported differences (different letters).*

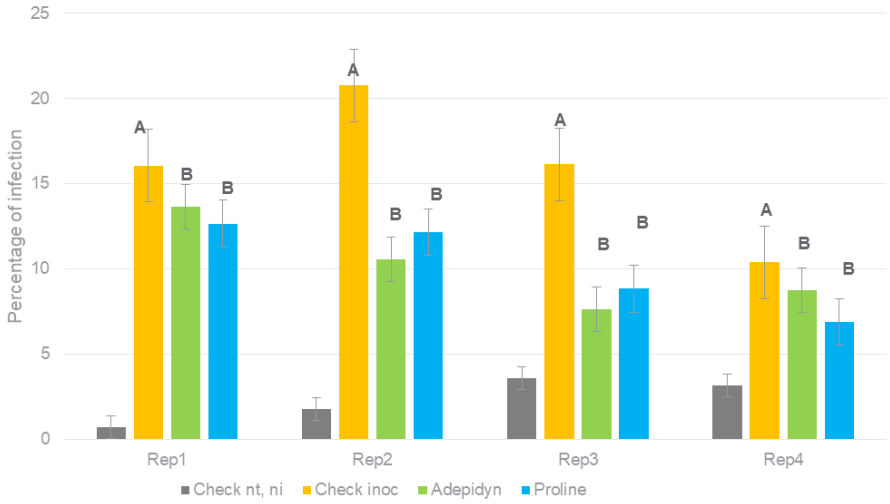

Figure 7: Percentage of infection of F. verticillioides *in the different replication plots and for the four treatments. Means with standard deviation are presented, together with information on statistically supported differences (different letters).*

Table 8: toxin analysis and fungal biomass µg/g in corn seeds

	F. graminearum		***F. verticillioides***	
	DON ppb	Fungal biomass	Fumonsin ppb	Fungal biomass
Check ni nt	724	0	17086.5	973.75
Check i	14732	10314	82749.25	3969.75
Adepidyn	16446	7275.5	77584.25	3978.25
Proline	14797.75	11368	75181.5	4093.63

- **Assay 2019**

Infection on ears for both pathogens (Fig.8A, B) were successful with more than 30% of symptoms for *F. graminearum* and 23% for *F. verticillioides.* Later treatment timing at R1 showed a trend of decrease of symptoms for *F. graminearum* (could not be statistically confirmed), and a similar efficacy for APN (25%) compared to Proline (26%). At an earlier treatment timing at V8-V10 APN showed also a trend for a better efficacy (30%) compared to Proline (40%). However, R1 treatments decreases symptoms of 5% compared to the check inoculated.

Adepidyn treatments showed no decrease in symptoms on ears infected with F. verticillioides compared to the check inoculated (23%). A later application timing (R1) with Proline showed a trend of a decrease of the symptoms (15%) compared to V8-V10 applications (20%).

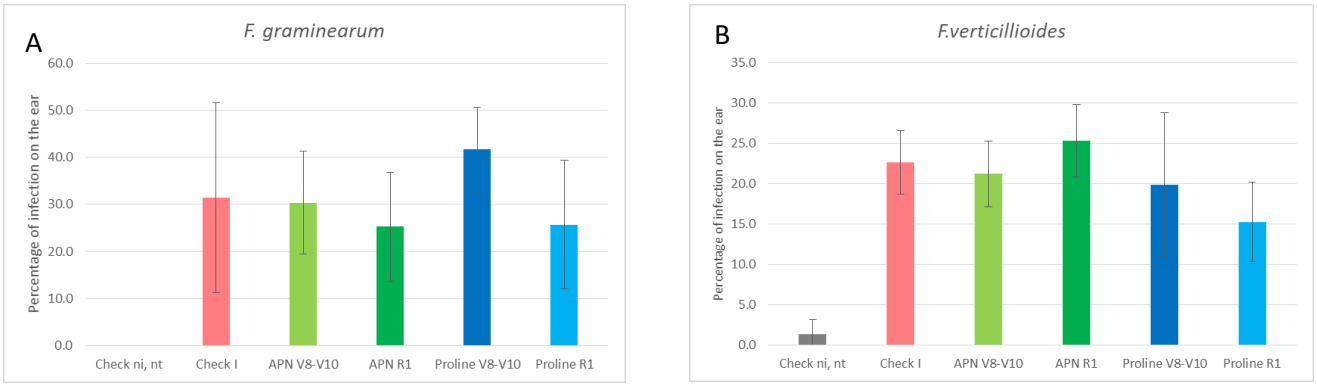

Figure 8: A. percentage of infection on the ear by F. graminearum*. B. Percentage of infection on the ears by* F. verticillioides*. Means with standard deviation are presented.*

5.4. Discussion

During all three years the influence of weather conditions on infections allowed an external contamination. It has been shown that temperature can influence infection, growth and mycotoxin production in wheat [30]. Infection methods for wheat assay were tested in the greenhouse and were workable in the field even if they were time consuming for a large amount of ears. This method allowed us to control the amount of spore suspension injected in each spikelet.

Each assay was assessed two to three times with 7 days interval and for the analysis one assessment time point was used. It was selected by considering the moment of external infection and of ear drying out. The curative assay on wheat showed a stronger infection level in check plots which might be because the inoculation was done few days earlier than for the preventive assay. It was shown in greenhouse that the infection pressure should not be influenced within 12 days after beginning of flowering time but in field conditions it might be influenced. Trial conditions were difficult in 2018 for wheat with very high temperature at inoculation which made it difficult for artificial point inoculation of spikelets (>30°C at 18 h). A few days after the inoculation there was a natural rain event presumably with a lot of natural infection. Artificial infection could not be differentiated from natural infection at late evaluation timing which often happening fact in field assays [112]. Preventive assay failed. Preventive vs curative assay could not be compared in 2018 due to the inappropriate weather conditions. The curative assay showed significant FHB effect of artificial inoculation at 21 dai only. Before the infection was too low (relative short time after infection and hot weather) and after 21 dai the natural infection was too high making it impossible to differentiate from the artificial inoculation. At 21 dai, all fungicides had a significant effect on visual disease development and the ranking of the products is: APN (ec) ≥ APN (sc) = proline ≥ caramba. It is known that prothioconazole (proline) induces hydrogen peroxide which triggers DON production by *F. graminearum* [76]. Wheat field assay in 2019 was performed with another wheat variety (Baretta instead of Tapidor in 2017 and 2018) and only two assessments could be done. Baretta and Tapidor are both resistant to rust but sensitive to *Fusarium*. Otherwise Baretta was tested in greenhouse and the same *F. graminearum* mixture was inoculated. There was no infection pressure differences to Tapidor. Very hot temperatures and sunny days lead to a faster drying of the plants. For the 21 dai assessment the symptoms could not be differentiated any more from the drying. For the curative assay disease control showed a ranking of the products as followed: APN EC > prothioconazole > APN SC > metconazole and after making the average of the three years the same ranking is confirmed. *F. graminearum* has proven so far difficult to control through the use of fungicides [31].

The average for TGW was done in the analysis but it must be taking in account that the variety Baretta has fewer heavy grains but the trend was kept by showing that APN EC increased TGW if compared to untreated. APN is known to have positive effects on Septoria disease which allows to keep green leaves longer and photosynthesis for grain filling. This could explain that treated grains with APN are heavier than uninoculated. Adepidyn could also reduce DON amount and showed the best results: APN EC > proline > APN SC > caramba. The preventive assay showed a trend for better disease control with APN EC but could not be significantly differentiated. TGW was higher for grains treated with APN EC and DON contamination showed following ranking: APN EC= APN SC= caramba > Proline. The 2018 field trials are similar to field trial results obtained in 2017. TGW was best for Adepidyn. This is likely due to the excellent foliar fungicide activity as compared to both Proline and caramba.

Significant differences could be observed for toxins and fungal biomass. Ranking of the products for DON control is also APN EC > prothioconazole > APN SC > metconazole. It is not shown in the analysis but 2019 assay was threatened by an external infection as toxin analysis revealed high T2/HT2 amounts which cannot be produced by F. *graminearum* isolates used in this study (confirmed by in vitro toxin analysis). A concurrence between DON and T2/HT2 might occurred which could explain low levels of DON in check plots.

In corn assays each year a different variety was used. ES Metronom, Avenir and Talisman and they were all resistant against Helminthosporium but sensitive to Fusarium. Starch amount in all of them is high and all are stabile at vegetation timing and by harvest. Plot design was different each year but did not influence the infection. The conditions were humogen all three years with no shadow, same seasonal timing, and each assay was rounded by blank plots. Otherwise spore suspensions used were not contaminated and irrigation was performed after inoculation and all maturing period. First year of corn assay did not went out because of the bad ear quality. Spathes opened early after infection which lead to bird attacks and grains were eaten by them. No test assay could be done in 2016 for selecting an optimal variety. 2018 and 2019 assays were performed using variety selected in a separate assay. F. *verticillioides* isolates used for these assays were also selected in separate assays. The infection was good against both Fusarium species: F. *verticillioides* and F. *graminearum* check reached 26%, and very low natural infection occurred. F. *graminearum* showed very good FHB symptoms but there was no significant differences between the treatments neither in the symptoms nor in the toxins. There was no differences between the efficacy of APN and PTZ. F. *verticillioides* showed also very good symptoms but there was no significant differences between the treatments neither in the symptoms nor in the toxins. There was no differences between the efficacy of APN and PTZ.

In 2019 application timing was studied by making the application of fungicides at two different timing, R1 and V8-V10. Results showed a trend for later application to control *F. graminearum* with Adepidyn. This might be the case because of treatments and inoculation timings which are closer to each other. We know from different assays the efficacy of APN against the same isolates used in corn. First In *vitro* data showed sensitivity to APN and proline for all strains used in the field assay. Secondly greenhouse assay (preventive and curative assay wheat) showed an efficacy of APN on the *F. graminearum* mixture also used in corn. Finally, field results from wheat assay showed also significant efficacy against the *F. graminearum* mixture. The reasons for a better visual efficacy of both fungicides on *F. verticillioides* might include different issues. First of all, the timing of application: preventive 2 days for F. *graminearum* and preventive 9 days for *F. verticillioides.* Products may need more time to get into the ear. Moreover, the inoculum was higher for *F. graminearum* (spore suspension) than for *F. verticillioides* (milled infected seeds with the pathogen). The application of these fungicides might be done earlier, before flowering, for a better control of *F. graminearum.* However, *F. verticillioides* infection is done on ears reaching 20 cm long. Further assays should be done to test if a later application on full developed ears would decrease fungal attack and application timing should be adapted before or after the infections.

6. Conclusions and outlook

The goal of this thesis was to understand how Fusarium head blight, a major plant disease complex and major pest in agriculture, can be better managed.

In Chapter 2, we could show by *in vitro* assays that fungicide sensitivity of *Fusarium* is very variable among species. The fungicide pydiflumetofen (SDHI) showed strong and best efficacy on isolates even if prothioconazole-desthio (DMI) was as good for some species. Interestingly sensitivities of *Fusarium* species to all available SDHIs were very specific and species-dependent, with existing cross resistance among fungicides. The work also showed that so far there is no resistance in *Fusarium* to pydiflumetofen.

Molecular analysis on *SDH* genes of Chapter 3 revealed unique gene identity of each species that allows to identify a species through its *SDH* genes. Clustering of the different species was preserved. These foundings might explain the differences observed in fungicide sensitivities to SDHIs.

Chapters 4 and 5 focused on plant protection by fungicide application. I could show that there is a fair amount of flexibility in fungicide application timing with adepidyn (pydiflumetofen) for the control of *Fusarium* and mycotoxin production. Preventive (up to 9 days before inoculation) and curative (12 days after inoculation) treatments were successful in the greenhouse. Also, the control of *Fusarium* and its mycotoxins in the field with adepidyn was convincing. However, here abiotic and biotic factors influenced fungicide effects. FHB control on corn with adepidyn was not effective, and procedures to improve the timing and methods of application need to be developed.

Dynamic reaction of FHB on chemical control have to be investigated in particular for APN usage. APN seems to control each species of the FHB complex but the selection of a less sensitive species have to be avoided.

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''Recognition is the most beautiful flower that springs from the soul'' Henry Ward Beecher.

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Appendix

Table 1: *Fusarium* isolates used in the study. CSN numbers of each isolate, origin, identification, provider, date of isolation, growth on petri dish, sporulation

CSN	original_ID	EPPO code	species_origin	species_ITS sequencing	Country	GPS	GPS 2	Host	host tissue	isolate provider	Origine	Date of reception	year of isolation	Sporulation	Growth on PDA plate
CS-FU00001	1201	FUSATI	tricinctum	tricinctum	France			barley	seed	IFBM		01.12.2016	2012	very good	very good
CS-FU00002	2103	FUSATI	tricinctum	tricinctum	France			barley	seed	IFBM	Lorraine	01.12.2016	2014	very good	very good
CS-FU00003	2104	FUSATI	tricinctum	tricinctum	France			barley	seed	IFBM	45	01.12.2016	2014	very good	very good
CS-FU00004	2105	FUSATI	tricinctum	tricinctum	France			barley	seed	IFBM	Yonne	01.12.2016	2014	very good	very good
CS-FU00005	2106	FUSATI	tricinctum	tricinctum	France			barley	seed	IFBM	Loiret	01.12.2016	2014	very good	very good
CS-FU00006	2115	FUSATI	tricinctum	tricinctum	France			barley	seed	IFBM	52	01.12.2016	2014	very good	very good
CS-FU00007	2116	FUSATI	tricinctum	tricinctum	France			barley	seed	IFBM	Côte d'or	01.12.2016	2014	very good	very good
CS-FU00008	2155	FUSACW	tricinctum	cerealis	France			barley	seed	IFBM		01.12.2016	2015	very good	very good
CS-FU00009	2156	FUSATI	tricinctum	tricinctum	France			barley	seed	IFBM		01.12.2016	2015	very good	very good
CS-FU00010	2157	GIBBZE	tricinctum	graminearum	France			barley	seed	IFBM		01.12.2016	2015	very good	very good
CS-FU00011	Fus2014-FR-001	GIBBZE	tricinctum	graminearum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00012	Fus2014-FR-002	GIBBZE	tricinctum	graminearum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00013	Fus2014-FR-003	FUSACW	tricinctum	cerealis	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00014	Fus2015-FR-017	FUSACW	tricinctum	cerealis	France			barley	seed	SYN FR Gyancourt		01.12.2016	2015	very good	very good
CS-FU00015	Fus2015-FR-018	FUSACW	tricinctum	cerealis	France			barley	seed	SYN FR Gyancourt		01.12.2016	2015	very good	very good
CS-FU00016	Fus2015-FR-019	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2015	bad	very good
CS-FU00017	Fus2014-FR-027	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good

CS-FU00018	Fus2014-FR-028	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00019	Fus2014-FR-029	GIBBZE	tricinctum	graminearum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00020	Fus2014-FR-030	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00021	Fus2014-FR-031	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00022	Fus2014-FR-032	GIBBZE	tricinctum	graminearum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00023	Fus2014-FR-033	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	bad	very good
CS-FU00024	Fus2014-FR-034	FUSACU	tricinctum	culmorum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00025	Fus2014-FR-035	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00026	Fus2014-FR-036	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00027	Fus2014-FR-037	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00028	Fus2014-FR-038	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00029	Fus2014-FR-044	GIBBZE	tricinctum	graminearum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00030	Fus2014-FR-045	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00031	Fus2014-FR-047	FUSATI	tricinctum	tricinctum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00032	Fus2014-FR-048	GIBBZE	tricinctum	graminearum	France			barley	seed	SYN FR Gyancourt		01.12.2016	2014	very good	very good
CS-FU00033	12FusF01.12	GIBBZE	tricinctum	graminearum	France	48.81329	7.584205	wheat	seed	France	Alsace	07.08.2012	2012	good	very good
CS-FU00034	12FusD09.02	FUSATI	tricinctum	tricinctum	Germany	51.83116	7.378349	wheat	seed	Germany	Nordrhein-Westfalen	10.08.2012	2012	very good	very good
CS-FU00035	12FusD09.15	FUSATI	tricinctum	tricinctum	Germany	51.83116	7.378349	wheat	seed	Germany	Nordrhein-Westfalen	10.08.2012	2012	bad	very good
CS-FU00036	14FusDE13.08	FUSATI	tricinctum	tricinctum	Germany	52.24287	8.42296	wheat	seed	Germany	Niedersachsen	20.08.2014	2014	bad	very good
CS-FU00037	14FusDK04.01	FUSATI	tricinctum	tricinctum	Denmark	55.53456	9.81675	wheat	seed	Danemark	Region Syddanmark	06.10.2014	2014	good	very good

CS-FU00038	14FusDK04.02	FUSATI	tricinctum	tricinctum	Germany	55.53456	9.81675	wheat	seed	Germany	Region Syddanmark	06.10.2014	2014	bad	very good
CS-FU00039	13FusD07.10	FUSATI	tricinctum	tricinctum	Germany	51.67863	12.86438	wheat	seed	Germany	Sachsen-Anhalt	22.08.2013	2013	bad	very good
CS-FU00040	13FusD08.02	FUSATI	tricinctum	tricinctum	Germany	49.73029	7.97819	wheat	seed	Germany	Rheinland-Pfalz	30.08.2013	2013	bad	very good
CS-FU00041	13FusD08.04	FUSATI	tricinctum	tricinctum	Germany	49.73029	7.97819	wheat	seed	Germany	Rheinland-Pfalz	30.08.2013	2013	bad	very good
CS-FU00042	13FusD11.02	FUSATI	tricinctum	tricinctum	Germany	52.36195	9.46101	wheat	seed	Germany	Niedersachsen	19.09.2012	2013	very good	very good
CS-FU00043	13FusD15.11	FUSATI	tricinctum	tricinctum	Germany	53.39551	8.02104	wheat	seed	Germany	Niedersachsen	30.08.2013	2013	good	very good
CS-FU00044	13FusD25.01	FUSATI	tricinctum	Verticillioides	Germany	53.84653	11.18732	wheat	seed	Germany	Mecklenburg-Vorpommern	07.08.2013	2013	very good	very good
CS-FU00045	13FusPL04.02	FUSATI	tricinctum	tricinctum	Poland	54.15015	16.2539	wheat	seed	Poland	Województwo zachodniopomorskie	05.12.2013	2013	bad	very good
CS-FU00046	13FUsSK09.01	FUSATI	tricinctum	tricinctum	Ukraine	48.00399	18.65556	wheat	seed	Ukraine	Nitra Region	25.07.2013	2013	very good	very good
CS-FU00047	13FusUK17.09	FUSATI	tricinctum	tricinctum	Ukraine	50.79844	17.5596	wheat	seed	Ukraine	England	05.11.2013	2013	very good	very good
CS-FU00048	14FusPL15.03	FUSATI	tricinctum	tricinctum	Poland	48.63142	8.03883	wheat	seed	Poland	opolskie	22.10.2014	2014	very good	very good
CS-FU00049	14FusPl_DE19.02	FUSATI	tricinctum	tricinctum	Germany	55.53456	9.81675	wheat	plants	Germany	Baden-Württemberg	14.03.2014	2014	good	very good
CS-FU00050	14FusPl_DK05.01	GIBBAV	tricinctum	avenaceum	Danemark			wheat	plants	Danemark	Region Syddanmark	25.03.2014	2014	good	very good
CS-FU00051		GIBBAC	Sporotrichoides	poae										good	very good
CS-FU00052	1025	FUSASP	Sporotrichoides	poae	France			barley		IFBM		01.09.2012	2012	very good	very good
CS-FU00053	1124	FUSASP	Sporotrichoides	sporotrichoides	France			barley		IFBM		01.09.2012	2012	very good	very good
CS-FU00054	1125	FUSALA	Sporotrichoides	langsethiae	France			barley		IFBM		01.10.2012	2012	bad	good
CS-FU00055	1209	FUSASP	Sporotrichoides	sporotrichoides	France			barley		IFBM	Seine et	01.12.2013	2013	bad	good

												Marne				
CS-FU00056	1931	FUSASP	Sporotrichioides	sporotrichoides	France			barley		IFBM	Seine et Marne	01.12.2013	2013	very good	very good
CS-FU00057	1932	FUSASP	Sporotrichioides	sporotrichoides	France			barley		IFBM		01.09.2014	2014	very good	very good
CS-FU00058	2015	FUSASP	Sporotrichioides	sporotrichoides	France			barley		IFBM		01.10.2014	2014	very good	very good
CS-FU00059	2059	FUSALA	Sporotrichioides	langsethiae	France			barley		IFBM	Bourgogne	01.10.2014	2014	very good	very good
CS-FU00060	2060	FUSALA	Sporotrichioides	langsethiae	France			barley		IFBM		01.10.2014	2014	very good	very good
CS-FU00061	2091	FUSALA	Sporotrichioides	langsethiae	France			barley		IFBM		01.11.2015	2015	very good	very good
CS-FU00062	2170	FUSASP	Sporotrichioides	sporotrichoides	France			barley		IFBM		02.07.2001	2010	very good	very good
CS-FU00063	10FusD01.1	FUSASP	Sporotrichioides	sporotrichoides	Germany	51.16569	10.45153	winter_wheat	seed	D			2010	bad	good
CS-FU00064	10FusD01.2	FUSASP	Sporotrichioides	sporotrichoides	Germany	51.16569	10.45153	winter_wheat	seed	D			2011	bad	good
CS-FU00065	11FusPL01.1	FUSASP	Sporotrichioides	sporotrichoides	Poland	51.22753	22.52869	winter_wheat	seed	PL	Lublin		2010	very good	very good
CS-FU00066	10FusPL01.2	FUSASP	Sporotrichioides	sporotrichoides	Poland	51.91944	19.14514	winter_wheat	seed	PL	Lublin		2010	very good	very good
CS-FU00067	10FusPL01.1	FUSASP	Sporotrichioides	sporotrichoides	Poland	51.91944	19.14514	winter_wheat	seed	PL			2011	very good	very good
CS-FU00068	11FusPL04	FUSASP	Sporotrichioides	sporotrichoides	Poland	51.22753	22.52869	winter_wheat	seed	PL			2012	very good	very good
CS-FU00069	12FusF01.18	FUSASP	Sporotrichioides	sporotrichoides	France	48.81329	7.584205	winter_wheat	seed	F	Alsace		2012	very good	very good
CS-FU00070	12FusD13.15	FUSASP	Sporotrichioides	sporotrichoides	Germany	51.13282	13.48332	winter_wheat	seed	D	Sachsen		2014	very good	very good
CS-FU00071	14FusDE20.03	FUSASP	Sporotrichioides	sporotrichoides	Germany	51.023	14.59161	winter_wheat	seed	D	Sachsen		2014	very good	very good
CS-FU00072	14FusDE21.06	FUSASP	Sporotrichioides	sporotrichoides	Germany	51.10774	14.60035	winter_wheat	seed	D	Sachsen		2015	very good	very good
CS-FU00073	15FusFR19.06	FUSASP	Sporotrichioides	tricinctum	France	49.74592	2.64709	wheat	seed	F	Picardie		2013	very good	very good
CS-FU00074	13FusSK10.02	FUSASP	Sporotrichioides	sporotrichoides	Slovakia	48.04709	18.65457	winter_wheat	seed	SK	Nitra			very good	very good
CS-FU00075	1203	FUSALA	langsethiae	langsethiae	France			barley		IFBM			2012	very good	very good
CS-FU00076	1210	FUSALA	langsethiae	langsethiae	France			barley		IFBM			2012	very good	very good

CS-FU00077	1129	FUSALA	langsethiae	langsethiae	France	barley	IFBM	2012	very good	very good
CS-FU00078	1737	FUSALA	langsethiae	langsethiae	France	barley	IFBM	2013	very good	very good
CS-FU00079	1739	FUSALA	langsethiae	langsethiae	France	barley	IFBM	2013	very good	very good
CS-FU00080	2019	FUSALA	langsethiae	langsethiae	France	barley	IFBM	2014	very good	very good
CS-FU00081	2056	FUSALA	langsethiae	langsethiae	France	barley	IFBM	2014	very good	very good
CS-FU00082	2129	FUSALA	langsethiae	langsethiae	France	barley	IFBM	2015	very good	very good
CS-FU00083	2130	FUSALA	langsethiae	langsethiae	France	barley	IFBM	2015	very good	very good
CS-FU00084	2131	FUSALA	langsethiae	langsethiae	France	barley	IFBM	2015	very good	very good
CS-FU00085		FUSASP	Sporotrichoides	sporotrichoides					very good	very good
CS-FU00086		FUSASP	Sporotrichoides	sporotrichoides					very good	very good
CS-FU00087		FUSASP	Sporotrichoides	sporotrichoides					very good	very good
CS-FU00088		FUSASP	Sporotrichoides	sporotrichoides					very good	very good
CS-FU00089		FUSALA	langsethiae	langsethiae					very good	very good
CS-FU00090		FUSALA	langsethiae	langsethiae					very good	very good
CS-FU00091		FUSALA	langsethiae	langsethiae					very good	very good
CS-FU00092		FUSATI	tricinctum	tricinctum					very good	very good
CS-FU00093		FUSATI	tricinctum	tricinctum					very good	very good
CS-FU00094		FUSATI	tricinctum	tricinctum					very good	very good
CS-FU00095	2101	FUSAPO	Poae	poae	France	barley	IFBM		good	very good
CS-FU00096	2082	FUSAPO	Poae	poae	France	barley	IFBM		good	very good
CS-FU00097	2017	FUSAPO	Poae	poae	France	barley	IFBM		good	bad
CS-FU00098	2016	FUSAPO	Poae	poae	France	barley	IFBM		good	very good

CS-FU00099	2147	FUSAPO	Poae	poae	France	barley	IFBM			bad	very good
CS-FU00100	2149	FUSAPO	Poae	poae	France	barley	IFBM			good	very good
CS-FU00101	2102	FUSAPO	Poae	poae	France	barley	IFBM			very good	very good
CS-FU00102	2141	FUSAPO	Poae	poae	France	barley	IFBM		2015	very good	very good
CS-FU00103	2144	FUSAPO	Poae	poae	France	barley	IFBM			very good	very good
CS-FU00104	2145	FUSAPO	Poae	poae	France	barley	IFBM			bad	very good
CS-FU00105		FUSAPO	Poae	poae						good	very good
CS-FU00106		FUSAPO	Poae	poae						good	very good
CS-FU00107		FUSAPO	Poae	poae						bad	good
CS-FU00108		FUSAPO	Poae	poae						good	very good
CS-FU00109		FUSAPO	Poae	poae						very good	very good
CS-FU00110		FUSAPO	Poae	poae						good	very good
CS-FU00111		FUSAPO	Poae	poae						good	very good
CS-FU00112	2073	GIBBZE	graminearum	graminearum	France	barley	IFBM			good	very good
CS-FU00113	2160	GIBBZE	graminearum	graminearum	France	barley	IFBM			very good	very good
CS-FU00114	1117	GIBBZE	graminearum	graminearum	France	barley	IFBM			bad	very good
CS-FU00115	1763	GIBBZE	graminearum	graminearum	France	barley	IFBM			good	very good
CS-FU00116	1167	GIBBZE	graminearum	graminearum	France	barley	IFBM			bad	very good
CS-FU00117	2022	GIBBZE	graminearum	graminearum	France	barley	IFBM			good	very good
CS-FU00118	2071	GIBBZE	graminearum	graminearum	France	barley	IFBM			good	very good
CS-FU00119	2161	GIBBZE	graminearum	graminearum	France	barley	IFBM			very good	very good
CS-FU00120	2162	GIBBZE	graminearum	graminearum	France	barley	IFBM			good	very good

CS-FU00121	2163	GIBBZE	graminearum	graminearum	France	barley	IFBM			bad	good
CS-FU00122	2072	GIBBZE	graminearum	graminearum	France	barley	IFBM			very good	very good
CS-FU00123		GIBBZE	graminearum	graminearum					2002	good	very good
CS-FU00124		GIBBZE	graminearum	graminearum					2013	good	very good
CS-FU00125		GIBBZE	graminearum	graminearum					2012	very good	very good
CS-FU00126		GIBBZE	graminearum	graminearum						bad	good
CS-FU00127		GIBBZE	graminearum	graminearum						bad	very good
CS-FU00128		GIBBZE	graminearum	graminearum						good	very good
CS-FU00129		GIBBZE	graminearum	graminearum						bad	bad
CS-FU00130		GIBBZE	graminearum	graminearum						good	good
CS-FU00131		FUSACW	graminearum	cerealis						very good	very good
CS-FU00132		FUSAVR	Vert	Verticillioides						very good	very good
CS-FU00133		FUSAVR	Vert	Verticillioides						very good	good
CS-FU00134		FUSAVR	Vert	Verticillioides						bad	very good
CS-FU00135		FUSAVR	Vert	Verticillioides						very good	bad
CS-FU00136		FUSAVR	Vert	Verticillioides						very good	very good
CS-FU00137		FUSAVR	Vert	Verticillioides						good	very good
CS-FU00138		FUSAVR	Vert	Verticillioides						good	very good
CS-FU00139		FUSAVR	Vert	Verticillioides						very good	bad
CS-FU00140		FUSAVR	Vert	Verticillioides						very good	very good
CS-FU00141		FUSAVR	Vert	Verticillioides						very good	very good
CS-FU00142	1320	GIBBAV	avenaceum	avenaceum	France	Barley	IFBM	01.01.2017	2012	good	very good

CS-FU00143	11FusD01	FUSAPO	Poae	poae	Germany	48.90941	12.6923	Wheat	Seed			Bayern	05.09.2011	2011	very good	very good
CS-FU00144	11FusF02.6/11FusF03.3	FUSAPO	Poae	poae	Switzerland	48.38267	7.614524	Wheat	Seed				21.07.2011	2011	very good	bad
CS-FU00145	12FusCH07.01	FUSAPO	Poae	poae	Germany	46.32975	6.923155	winter_wheat	seed	Les Barges		Wallis		2012	bad	very good
CS-FU00146	12FusD06.01	FUSAPO	Poae	poae	Germany	53.10824	8.46917	winter_wheat	seed	27798 Hude		Niedersachsen	13.08.2012	2012	very good	very good
CS-FU00147	12FusD10.02	FUSAPO	Poae	Poae	Germany			winter_wheat	seed	50170 Kerpen-Buir		Nordrhein_Westfalen	02.08.2012	2012	bad	very good
CS-FU00148	12FusF02.16	FUSAPO	Poae	poae	Germany	48.81329	7.584205	winter_wheat	seed	67350 Ettendorf		Alsace	24.07.2012	2012	good	very good
CS-FU00149	13FusD14.03	FUSAPO	Poae	poae	Germany	51.18591	9.87328	winter_wheat	seed	01623 Lommatzsch		Sachsen	11.09.2013	2013	very good	very good
CS-FU00150	13FusD15.19	FUSAPO	Poae	poae	Germany	53.3951	8.02104	winter_wheat	seed	26345 Bockhold		Niedersachsen	30.08.2013	2013	good	very good
CS-FU00151	13FusPL01.01	FUSAPO	Poae	poae	Poland	51.25194	22.55349	winter_wheat	seed	74-110 Banie		West_Pomeranian	06.12.2013	2013	very good	very good
CS-FU00152	13FusPL05.03	FUSAPO	Poae	poae	Poland	54.12142	16.16863	winter_wheat	seed	76-024 Swieszyno		West_Pomeranian	05.12.2013	2013	very good	very good
CS-FU00153	14FusDE03.02	FUSAPO	Poae	poae	Germany	52.26015	9.97092	winter_wheat	seed	31191 Hannover		Niedersachsen	04.09.2014	2014	very good	very good
CS-FU00154	14FusDE05.03	FUSAPO	Poae	poae	Germany	53.84532	11.48314	winter_wheat	seed	23972 Dorf Mecklenburg		Mecklenburg-Vorpommern	12.08.2014	2014	good	very good
CS-FU00155	14FusDK02.01	FUSAPO	Poae	poae	Germany	55.50915	9.032865	winter_wheat	seed	6650 Brorup		Syddanmark	06.10.2014	2014	very good	very good
CS-FU00156	14FusPL01.01	FUSAPO	Poae	poae	Poland	50.26874	18.53738	winter_wheat	seed	44-153 Sosnicowice		Silesia	22.10.2014	2014	very good	very good
CS-FU00157	14FusPL05.05	FUSAPO	Poae	poae	Poland	50.84447	23.92572	winter_wheat	seed	22-500 Hrubieszow		Lublin	22.10.2014	2014	bad	very good
CS-FU00158	14FusPL10.09	FUSAPO	Poae	poae	Poland	52.18517	19.79668	winter_wheat	seed	99-440 Zduny		Lodz	22.10.2014	2014	bad	very good
CS-FU00159	15FusDE02.01	FUSAPO	Poae	poae	Germany	48.52194	9.79194	Winter_wheat	seed	89191 Nellingen-Aichen		Baden-Württemberg	17.11.2015	2015	very good	very good
CS-FU00160	15FusDE05.03	FUSAPO	Poae	poae	Germany	49.84318	8.467169	Winter_wheat	seed	64560 Riedstadt-Leeheim		Hessen	17.11.2015	2015	very good	very good
CS-FU00161	15FusDE14.06	FUSAPO	Poae	poae	Germany	50.62589	7.03195	Winter_wheat	seed	53340 Meckenheim		Nordrhein_Westfalen	17.11.2015	2015	very good	very good

CS-FU00162	15FusDE25.02	FUSAPO	Poae	poae	Germany	49.96931	12.16465	Winter_wheat	seed	93083 Obertraubling	Bayern	17.11.2015	2015	good	good
CS-FU00163	15FusDE25.04	FUSAPO	Poae	poae	Germany	49.96931	12.16465	Winter_wheat	seed	93083 Obertraubling	Bayern	17.11.2015	2015	bad	bad
CS-FU00164	15FusFR21.01	FUSALA	Poae	langsethiae	France	49.74592	2.6 4709	Wheat	seed	80910 Arvillers	Picardie	15.12.2015	2015	good	good
CS-FU00165	16FusI01.03	FUSAPO	Poae	poae	Italy	44.73457	11.69082		seed	44124 Ferrara	Emilia-Romagna	07.07.2016	2016	very good	good
CS-FU00166	16FusUA02.04	FUSAPO	Poae	poae	Austria	49.72045	3.0 1166		seed	9100 Bila Tserkva	oblast_kiev	29.07.2016	2016	very good	very good
CS-FU00167	16FusUA04.04	FUSAPO	Poae	poae	Austria	49.72045	3.0 1166		seed	9100 Bila Tserkva	oblast_kiev	29.07.2016	2016	very good	very good
CS-FU00168	16FusUA05.07	FUSAPO	Poae	poae	Austria	49.72045	3.0 1166		seed	9100 Bila Tserkva	oblast_kiev	29.07.2016	2016	bad	good
CS-FU00169	16FusDE15.04	FUSAPO	Poae	poae	Germany	52.36785	9.3 1888	winter_wheat	seed	31699 Beckendorf	Niedersachsen	06.09.2016	2016	bad	good
CS-FU00170	16FusDE17.07	FUSAPO	Poae	poae	Germany	52.34725	9.3 21667	winter_wheat	seed	31542 Bad Nenndorf	Niedersachsen	06.09.2016	2016	bad	good
CS-FU00171	16FusSE03.03	FUSAPO	Poae	poae	Slovakia	58.48626	16.06047	Wheat	seed	61021 Norsholm	ostergotlands_ian	30.09.2016	2016	very good	very good
CS-FU00172	16FusFIN03.05	FUSAPO	Poae	poae	Finland	60.09382	23.84101	Spring_wheat	seed	10230 Inga	south_finland	07.11.2016	2016	very good	very good
CS-FU00173	13FusUK14.01	FUSAPO	Poae	poae	Unitedkingdom	51.1166	-1.4843	winter_wheat	seed	SO20 GRQ Stockbridge	South_East	30.09.2013	2013	very good	very good
CS-FU00174	13FusUK14.02	FUSAPO	Poae	poae	Unitedkingdom	51.1166	-1.4843	winter_wheat	seed	SO20 GRQ Stockbridge	South_East	30.09.2013	2013	very good	very good
CS-FU00175	13FusUK14.03	FUSAPO	Poae	poae	Unitedkingdom	51.1166	-1.4843	winter_wheat	seed	SO20 GRQ Stockbridge	South_East	30.09.2013	2013	very good	very good
CS-FU00176	13FusUK15.03	FUSAPO	Poae	poae	Unitedkingdom	52.62416	0.4 60131	winter_wheat	seed	PE33 9MA Stradsett	East_of_England	01.11.2013	2013	very good	very good
CS-FU00177	13FusUK15.07	FUSAPO	Poae	poae	Unitedkingdom	52.62416	0.4 60131	winter_wheat	seed	PE33 9MA Stradsett	East_of_England	01.11.2013	2013	very good	very good
CS-FU00178	13FusUK15.08	FUSAPO	Poae	poae	Unitedkingdom	52.62416	0.4 60131	winter_wheat	seed	PE33 9MA Stradsett	East_of_England	01.11.2013	2013	very good	very good
CS-FU00179	13FusUK15.13	FUSAPO	Poae	poae	Unitedkingdom	52.62416	0.4 60131	winter_wheat	seed	PE33 9MA Stradsett	East_of_England	01.11.2013	2013	very good	very good
CS-FU00180	13FusUK16.01	FUSAPO	Poae	poae	Unitedkingdom	52.85005	1.0 49813	winter_wheat	seed	NR24 2ER Melton Constable	East_of_England	31.10.2013	2013	very good	very good
CS-FU00181	14FusPl_CZ05.05	FUSAPO	Poae	poae	Tchechien	49.22474	17.54195	winter_wheat	seed	75621 Katerinice	Southern_Moravia	28.03.2014	2014	very good	very good

CS-FU00182	14FusPl_CZ05.10	FUSAPO	Poae	poae	Tchechien	49.22474	17.54195	winter_wheat	seed	75621 Katerinice	Southern_Moravia	28.03.2014	2014	very good	very good
CS-FU00183	10FusD02.1	FUSAPO	Poae	poae	Germany	51.16569	10.45153	Wheat	seed				2010	bad	very good
CS-FU00184	10FusD02.2	FUSAPO	Poae	poae	Germany	51.16569	10.45153	Wheat	seed				2010	bad	very good
CS-FU00185	11FusDK02.1	FUSAPO	Poae	poae	Denmark	56.26392	9.501785	Wheat	Seed		Sjaelland	04.10.2011	2011	bad	very good
CS-FU00186	11FusDK02.2	FUSAPO	Poae	poae	Denmark	56.26392	9.501785	Wheat	Seed		Sjaelland	04.10.2011	2011	bad	very good
CS-FU00187	11FusDK03.1	FUSAPO	Poae	poae	Denmark	55.92608	11.66539	Wheat	Seed		Sjaelland	04.10.2011	2011	very good	very good
CS-FU00188	11FusDK03.2	FUSAPO	Poae	poae	Denmark	55.92608	11.66539	Wheat	Seed		Sjaelland	04.10.2011	2011	very good	very good
CS-FU00189	11FusF02.1	FUSAPO	Poae	poae	France	48.38267	7.614524	Wheat	Seed		Alsace	21.07.2011	2011	very good	very good
CS-FU00190	11FusF02.6/11FusF03.3	FUSAPO	Poae	poae	France	48.38267	7.614524	Wheat	Seed		Alsace	21.07.2011	2011	very good	very good
CS-FU00191	11FusF02.7	FUSATRI	Poae	tricinctum	France	48.38267	7.614524	Wheat	Seed		Alsace	21.07.2011	2011	very good	very good
CS-FU00192	11FusF02.9	FUSATRI	Poae	tricinctum	France	48.38267	7.614524	Wheat	Seed		Alsace	21.07.2011	2011	very good	very good
CS-FU00193	11FusF03.1	FUSAPO	Poae	poae	France	48.38267	7.614524	Wheat	Seed		Alsace	21.07.2011	2011	very good	very good
CS-FU00194	11FusF03.2	FUSAPO	Poae	poae	France	48.38267	7.614524	Wheat	Seed		Alsace	21.07.2011	2011	very good	very good
CS-FU00195	2097	GIBBAV	avenaceum	avenaceum	France	48.38267	7.614524	Barley				21.07.2011		very good	very good
CS-FU00196	11FusF03.4	FUSAPO	Poae	poae	France	48.38267	7.614524	Wheat	Seed		Alsace	21.07.2011	2011	very good	very good
CS-FU00197		FUSAPO	Poae	poae	unknown									very good	very good
CS-FU00198	4850-a	GIBBZE	graminearum	graminearum	Hungary			Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00199	4850-b	GIBBZE	graminearum	graminearum	Hungary			Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00200	4850-c	GIBBZE	graminearum	graminearum	Hungary			Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00201	4850-d	GIBBZE	graminearum	graminearum	Hungary			Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good

CS-FU00202	4850-e	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00203	4850-f	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00204	3851-a	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00205	3851-b	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00206	3851-c	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00207	3851-d	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00208	3851-e	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00209	4872-a	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00210	4872-b	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00211	4872-c	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00212	4872-d	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00213	4872-e	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00214	4872-f	FUSAVR	graminearum	Verticillioides	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00215	4856-a	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00216	4856-b	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00217	4856-c	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00218	4856-d	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00219	4856-e	GIBBZE	graminearum	graminearum	Hungary	Zea mays	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good

						[la;XX; 1]							
CS-FU00220	4856-f	GIBBZE	graminearum	graminearum	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00221	4850-g	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00222	4850-h	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00223	4850-i	FUSAPF	liseola sub	proliferatum	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00224	4850-j	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00225	4850-k	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00226	4850-l	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Debrecen	16.10.2017	2017	very good	very good
CS-FU00227	3851-f	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00228	3851-g	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00229	3851-h	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00230	3851-i	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00231	3851-j	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Szeged	16.10.2017	2017	very good	very good
CS-FU00232	4872-g	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00233	4872-h	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00234	4872-i	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00235	4872-j	FUSAPF	liseola sub	proliferatum	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00236	4872-k	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX; 1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good

CS-FU00237	4872-l	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Boly	16.10.2017	2017	very good	very good
CS-FU00238	4856-g	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00239	4856-h	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00240	4856-i	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00241	4856-j	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00242	4856-k	FUSAVR	liseola sub	Verticillioides	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00243	4856-l	FUSAPF	liseola sub	proliferatum	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00244	4856-m	GIBBFS	liseola sub	subglutinans	Hungary	Zea mays [la;XX;1]	corn ear	Syngenta Lombez	Cegled	16.10.2017	2017	very good	very good
CS-FU00245	1318	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00246	2100	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00247	1915	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00248	1914	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00249	1913	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00250	2098	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00251	2099	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00252	2134	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00253	2097	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00254	1320	GIBBAV	avenaceum	avenaceum	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00255	1911	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM		01.12.2016		very good	very good
CS-FU00256	1999	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM		01.12.2016		very good	very good

CS-FU00257	2128	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00258	372	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00259	880	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00260	1019	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00261	1020	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00262	1060	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00263	1854	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00264	265	GIBBIN	equiseti	equiseti	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00265	1207	GIBBZE	culmorum	graminearum	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00266	1208	FUSACU	culmorum	culmorum	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00267	1586	FUSACU	culmorum	culmorum	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00268	2084	GIBBZE	culmorum	graminearum	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00269	1202	FUSACU	culmorum	culmorum	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00270	2083	FUSACW	culmorum	cerealis	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00271	2126	FUSACU	culmorum	culmorum	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00272	1924	FUSACU	culmorum	culmorum	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00273	2080	FUSACU	culmorum	culmorum	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00274	2083	FUSACW	culmorum	cerealis	France	Barley	Seed	IFBM	01.12.2016		very good	very good
CS-FU00275	11FusD17.2	FUSACU	culmorum	culmorum	Germany	Wheat	Seed			2011	very good	very good
CS-FU00276	12FusD04.105	GIBBZE	culmorum	graminearum	Germany	Wheat	Seed			2012	very good	very good
CS-FU00277	13FusD06.05	FUSACU	culmorum	culmorum	Germany	Wheat	Seed			2013	very good	very good
CS-FU00278	14FusDE21.10	FUSACU	culmorum	culmorum	Germany	Wheat	Seed			2014	very good	very good

CS-FU00279	14FusPl_CH1.02	FUSACU	culmorum	culmorum	Switzerland	Wheat	Seed	2014	very good	very good
CS-FU00280	14FusPl_DE01.12	FUSACW	culmorum	cerealis	Germany	Wheat	Seed	2014	very good	very good
CS-FU00281	14FusPl_DE06.01	FUSACU	culmorum	culmorum	Germany	Wheat	Seed	2014	very good	very good
CS-FU00282	14FusPl_DK03.01	FUSACU	culmorum	tricinctum	Danemark	Wheat	Seed	2014	very good	very good
CS-FU00283	14FusPl_DK04.01	FUSACW	culmorum	cerealis	Danemark	Wheat	Seed	2014	very good	very good
CS-FU00284	14FusPl_DK05.03	FUSACU	culmorum	culmorum	Danemark	Wheat	Seed	2014	very good	very good
CS-FU00285	14FusPl_DK05.04	GIBBAV	culmorum	avenaceum	Danemark	Wheat	Seed	2014	very good	very good
CS-FU00286	14FusPl_DK07.01	GIBBAV	culmorum	avenaceum	Danemark	Wheat	Seed	2014	very good	very good
CS-FU00287	14FusPl_FR01.01		culmorum	venenatum	France	Wheat	Seed	2014	very good	very good
CS-FU00288	14FusPl_FR01.05	FUSACW	culmorum	cerealis	France	Wheat	Seed	2014	very good	very good
CS-FU00289	14FusPl_FR04.01	GIBBZE	culmorum	graminearum	France	Wheat	Seed	2014	very good	very good
CS-FU00290	14FusPl_PO21.01	FUSACU	culmorum	culmorum	Poland	Wheat	Seed	2014	very good	very good
CS-FU00291	14FusPl_PO21.02	FUSACU	culmorum	culmorum	Poland	Wheat	Seed	2014	very good	very good
CS-FU00292	14FusPl_PO21.03	FUSACU	culmorum	culmorum	Poland	Wheat	Seed	2014	very good	very good
CS-FU00293		FUSACU	culmorum	culmorum			Seed	2013	very good	very good
CS-FU00294	14FusPl_PO22.04	FUSACU	culmorum	culmorum	Poland	Wheat	Seed	2014	very good	very good
CS-FU00295	14FusPl_PO23.02	FUSACU	culmorum	culmorum	Poland	Wheat	Seed	2014	very good	very good
CS-FU00296	14FusPl_PO23.03	GIBBZE	culmorum	avenaceum	Poland	Wheat	Seed	2014	very good	very good
CS-FU00297	14FusPl_PO23.04	GIBBAV	culmorum	avenaceum	Poland	Wheat	Seed	2014	very good	very good
CS-FU00298	14FusPl_PO23.05	GIBBIN	culmorum	equiseti	Poland	Wheat	Seed	2014	very good	very good
CS-FU00299	14FusPl_SE01.01	FUSACU	culmorum	culmorum	Slovakia	Wheat	Seed	2014	very good	very good
CS-FU00300	14FusPl_SE01.02	FUSACU	culmorum	culmorum	Slovakia	Wheat	Seed	2014	very good	very good

CS-FU00301	14FusPl_SE01.03		culmorum	venenatum	Slovakia	Wheat	Seed	2014	very good	very good
CS-FU00302	14FusPl_SE02.01	FUSACU	culmorum	culmorum	Slovakia	Wheat	Seed	2014	very good	very good
CS-FU00303	14FusPl_SE02.02	FUSACU	culmorum	culmorum	Slovakia	Wheat	Seed	2014	very good	very good
CS-FU00304	14FusPl_SE02.05	FUSACU	culmorum	culmorum	Slovakia	Wheat	Seed	2014	very good	very good
CS-FU00305	17FusPL_PO21.4	GIBBAV	culmorum	avenaceum	Poland	Wheat	Seed	2017	very good	very good
CS-FU00306	15FusFR05.01	GIBBAV	culmorum	avenaceum	France	Wheat	Seed	2015	very good	very good
CS-FU00307	15FusIRL03.01	FUSACW	culmorum	cerealis	Irland	Wheat	Seed	2015	very good	very good
CS-FU00308	15FusIRL04.02	FUSACU	culmorum	culmorum	Irland	Wheat	Seed	2015	very good	very good
CS-FU00309	16FusBE02.01	FUSACU	culmorum	culmorum	Belgium	Wheat	Seed	2016	very good	very good
CS-FU00310	16FusBE02.02	FUSACU	culmorum	poae	Belgium	Wheat	Seed	2016	very good	very good
CS-FU00311	16FusBE02.10	GIBBZE	culmorum	graminearum	Belgium	Wheat	Seed	2016	very good	very good
CS-FU00312	16FusDE01.02	FUSATRI	culmorum	tricinctum	Germany	Wheat	Seed	2016	very good	very good
CS-FU00313	16FusDE04.06	GIBBZE	culmorum	graminearum	Germany	Wheat	Seed	2016	very good	very good
CS-FU00314	16FusDE18.08	GIBBZE	culmorum	graminearum	Germany	Wheat	Seed	2016	very good	very good
CS-FU00315	16FusDK01.02	FUSATRI	culmorum	tricinctum	Danemark	Wheat	Seed	2016	very good	very good
CS-FU00316	16FusDK01.07	FUSACU	culmorum	culmorum	Danemark	Wheat	Seed	2016	very good	very good
CS-FU00317	16FusDK02.01	FUSACU	culmorum	culmorum	Danemark	Wheat	Seed	2016	very good	very good
CS-FU00318	16FusDK02.03	FUSATRI	culmorum	tricinctum	Danemark	Wheat	Seed	2016	very good	very good
CS-FU00319	16FusDK05.01	FUSATRI	culmorum	tricinctum	Danemark	Wheat	Seed	2016	very good	very good
CS-FU00320	16FusDK05.09	GIBBZE	culmorum	graminearum	Danemark	Wheat	Seed	2016	very good	very good
CS-FU00321	16FusDK07.06	FUSACU	culmorum	culmorum	Danemark	Wheat	Seed	2016	very good	very good
CS-FU00322	16FusI01.08	FUSACU	culmorum	culmorum	Italy	Wheat	Seed	2016	very good	very good

CS-FU00323	16FusI03.02	FUSAPO	culmorum	poae	Italy	Wheat	Seed				2016	very good	very good
CS-FU00324	16FusSE02.04	GIBBZE	culmorum	graminearum	Slovakia	Wheat	Seed				2016	very good	very good
CS-FU00325	16FusSE04.01	GIBBZE	culmorum	graminearum	Slovakia	Wheat	Seed				2016	very good	very good
CS-FU00326		FUSACU	culmorum	culmorum			Seed				2013	very good	very good
CS-FU00327		FUSACU	culmorum	culmorum			Seed				2012	very good	very good
CS-FU00328		GIBBZE	graminearum	graminearum			Seed					very good	very good
CS-FU00329		GIBBZE	graminearum	graminearum			Seed					very good	very good
CS-FU00330		GIBBZE	graminearum	graminearum			Seed					very good	very good
CS-FU00331	Fa01	GIBBAV	avenaceum	avenaceum	Germany	Barley	Seed	TUM	Thüringen	20.03.2018	2012	very good	very good
CS-FU00332	Fa02	GIBBAV	avenaceum	avenaceum	Germany	Barley	Seed	TUM	Freising	20.03.2018	2012	very good	very good
CS-FU00333	Fa002	FUSATRI	avenaceum	tricinctum	Germany	Barley	Seed	TUM	Sachsen	20.03.2018	2009	very good	very good
CS-FU00334	Fa001	GIBBAV	avenaceum	avenaceum	Germany	Barley	Seed	TUM	Rügen	20.03.2018	2009	very good	very good
CS-FU00335	Fa04	GIBBAV	avenaceum	avenaceum	Germany	Barley	Seed	TUM	Güstrow	20.03.2018	2014	very good	very good
CS-FU00336	Fa05	GIBBAV	avenaceum	avenaceum	Poland	Barley	Seed	TUM	Polen	20.03.2018	1998	very good	very good
CS-FU00337	Fa06	FUSACW	avenaceum	cerealis	Germany	Wheat	Seed	TUM	Grünbach	20.03.2018	1991	very good	very good
CS-FU00338	Fa07	GIBBZE	avenaceum	graminearum	Finland	Getreide	Seed	TUM	Finnland	20.03.2018	2001	very good	very good
CS-FU00339	Fc01	GIBBZE	culmorum	graminearum	Germany	Barley	Seed	TUM	Freising	20.03.2018	2012	very good	very good
CS-FU00340	Fc02	FUSACU	culmorum		Germany	Barley	Seed	TUM	Freising	20.03.2018	2012	bad	bad
CS-FU00341	Fc03	FUSATRI	culmorum	tricinctum	Poland	Barley	Seed	TUM	Polen	20.03.2018	2012	very good	very good
CS-FU00342	Fc002	FUSATRI	culmorum	tricinctum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2008	very good	very good
CS-FU00343	Fc04	FUSATRI	culmorum	tricinctum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2010	very good	very good

CS-FU00344	Fc06	FUS ATRI	culmorum	tricinctum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2010	very good	very good
CS-FU00345	Fc08	FUS ACU	culmorum	culmorum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2012	very good	very good
CS-FU00346	Fc09	GIBB ZE	culmorum	graminearum	France	Wheat	Seed	TUM	Frankreich	20.03.2018	1999	very good	very good
CS-FU00347	Fc10	FUS ACU	culmorum	culmorum	France	Wheat	Seed	TUM	Frankreich	20.03.2018	1999	very good	very good
CS-FU00348	Fc11	FUS ACU	culmorum	culmorum	France	Wheat	Seed	TUM	Frankreich	20.03.2018	1999	very good	very good
CS-FU00349	Fc12	FUS ACU	culmorum	culmorum	France	Wheat	Seed	TUM	Frankreich	20.03.2018	1999	very good	very good
CS-FU00350	Fc13	FUS ACU	culmorum	culmorum	Germany	Wheat	Seed	TUM	Deutschland	20.03.2018	1993	very good	very good
CS-FU00351	Fg01	FUS ACW	graminearum	cerealis	Germany	Barley	Seed	TUM	Freising	20.03.2018	2012	very good	very good
CS-FU00352	Fg006	GIBB ZE	graminearum	graminearum	Germany		Seed	TUM	Seefeld	20.03.2018	2010	very good	very good
CS-FU00353	Fg04	FUS ATRI	graminearum	tricinctum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2010	very good	very good
CS-FU00354	Fg05	GIBB ZE	graminearum		Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2009	bad	bad
CS-FU00355	Fg06	GIBB ZE	graminearum	graminearum	Germany	Trithordeum	Seed	TUM	Weihenstephan	20.03.2018	2009	very good	very good
CS-FU00356	Fg07	GIBB ZE	graminearum	graminearum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2009	very good	very good
CS-FU00357	Fg02	GIBB ZE	graminearum	culmorum	Germany	Barley	Seed	TUM	Güstrow	20.03.2018	2014	very good	very good
CS-FU00358	Fg03	GIBB ZE	graminearum	graminearum	Germany	Blatt, Apfel	Seed	TUM	Deutschland	20.03.2018	1996	very good	very good
CS-FU00359	Fg08	GIBB ZE	graminearum	graminearum	Germany	Wheat	Seed	TUM	Deutschland	20.03.2018		very good	very good
CS-FU00360	Fg09	FUS ACU	graminearum	culmorum	Niederlande	Getreide	Seed	TUM	Niederlande	20.03.2018	1964	very good	very good
CS-FU00361	Fp01	FUS ATRI	Poae	tricinctum	Germany	Gerstenmalz	Seed	TUM	Thüringen	20.03.2018	2012	very good	very good
CS-FU00362	Fp02	FUS ATRI	Poae	tricinctum	Germany	Gerste	Seed	TUM	Freising	20.03.2018	2012	very good	very good
CS-FU00363	Fp03	FUS ATRI	Poae	tricinctum	Poland	Gerste	Seed	TUM	Polen	20.03.2018	2012	very good	very good

CS-FU00364	Fp001	FUSATRI	Poae	tricinctum	Germany	Gerste	Seed	TUM	Deutschland	20.03.2018	2009	very good	very good
CS-FU00365	Fp04	FUSATRI	Poae	tricinctum	Germany	Gerste	Seed	TUM	Deutschland	20.03.2018	2001	very good	very good
CS-FU00366	Fp05	FUSAPO	Poae	poae	Germany	Gerste	Seed	TUM	Deutschland	20.03.2018	2001	very good	very good
CS-FU00367	Fp06	FUSAPO	Poae	poae	Finland	Gerste	Seed	TUM	Finnalnd	20.03.2018	1994	very good	very good
CS-FU00368	Fp07	FUSAPO	Poae	poae	unknown	Wheat	Seed	TUM		20.03.2018		very good	very good
CS-FU00369	Fs002	FUSASP	Sporotrichoides	sporotrichoides	unknown	Barley	Seed	TUM	Weihenstephan	20.03.2018	2009	very good	very good
CS-FU00370	Fs001	GIBBZE	Sporotrichoides	graminearum	Danemark	Juncus sp	Seed	TUM	Dänemark	20.03.2018	2009	very good	very good
CS-FU00371	Fs02	FUSATI	Sporotrichoides	tricinctum	Italy	corn	Seed	TUM	Italien	20.03.2018		very good	very good
CS-FU00372	Fs03	FUSASP	Sporotrichoides		Germany	Barley	Seed	TUM	Deutschland	20.03.2018	2010	bad	bad
CS-FU00373	Fs04	FUSASP	Sporotrichoides	sporotrichoides	Germany	Wheat	Seed	TUM	Deutschland	20.03.2018	2012	very good	very good
CS-FU00374	Fs05	FUSATI	Sporotrichoides	tricinctum	Germany	Barley	Seed	TUM	Deutschland	20.03.2018	2012	very good	very good
CS-FU00375	Ft001	FUSATI	tricinctum		Danemark	Barley	Seed	TUM	Dänemark	20.03.2018	2009	bad	bad
CS-FU00376	Ft01(a)	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Bayern	20.03.2018	2012	very good	very good
CS-FU00377	Ft01(b)	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Bayern	20.03.2018	2012	very good	very good
CS-FU00378	Ft02	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Thüringen	20.03.2018	2012	very good	very good
CS-FU00379	Ft05	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2009	very good	very good
CS-FU00380	Ft06	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2010	very good	very good
CS-FU00381	Ft07	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Weihenstephan	20.03.2018	2008	very good	very good
CS-FU00382	Ft08	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Deutschland	20.03.2018	2008	very good	very good
CS-FU00383	Ft09	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Deutschland	20.03.2018	2008	very good	very good
CS-FU00384	Ft10	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Deutschland	20.03.2018	2000	very good	very good

CS-FU00385	Ft11	FUSATI	tricinctum	tricinctum	Danemark	Barley	Seed	TUM	Dänemark	20.03.2018	1986	very good	very good
CS-FU00386	Ft12	GIBBAV	tricinctum	avenaceum	Finland	Barley	Seed	TUM	Finland	20.03.2018	1996	very good	very good
CS-FU00387	Fus01	FUSATI	tricinctum		Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	bad	bad
CS-FU00388	Fus02	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00389	Fus03	FUSATI	tricinctum	tricinctum	Sweden	Barley	Seed	TUM	Schweden	20.03.2018	2015	very good	very good
CS-FU00390	Fus04	FUSATI	tricinctum	tricinctum	Sweden	Barley	Seed	TUM	Schweden	20.03.2018	2015	very good	very good
CS-FU00391	Fus05	FUSATI	tricinctum	tricinctum	Sweden	Barley	Seed	TUM	Schweden	20.03.2018	2015	very good	very good
CS-FU00392	Fus06	FUSAPO	Poae	poae	Sweden	Barley	Seed	TUM	Schweden	20.03.2018	2015	very good	very good
CS-FU00393	Fus07	GIBBZE	culmorum	graminearum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00394	Fus08	FUSATRI	avenaceum	tricinctum	Germany	Barley	Seed	TUM	Sachsen-Anhalt	20.03.2018	2015	very good	very good
CS-FU00395	Fus09	FUSAPO	Sporotrichoides	poae	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00396	Fus10	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00397	Fus13	FUSATI	avenaceum	tricinctum	Germany	Barley	Seed	TUM	Thüringen	20.03.2018	2015	very good	very good
CS-FU00398	Fus14	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Thüringen	20.03.2018	2015	very good	very good
CS-FU00399	Fus15	GIBBAV	avenaceum	avenaceum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00400	Fus16	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00401	Fus17	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00402	Fus18	FUSAPO	Sporotrichoides	tricinctum	Germany	Barley	Seed	TUM	Schwaben	20.03.2018	2015	very good	very good

CS-FU00403	Fus19	FUSATI	tricinctum	tricinctum	Danemark	Barley	Seed	TUM	Dänemark	20.03.2018	2015	very good	very good
CS-FU00404	Fus20	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Bayern	20.03.2018	2015	very good	very good
CS-FU00405	Fus21	FUSATI	tricinctum, avenaceum	tricinctum	Danemark	Barley	Seed	TUM	Dänemark	20.03.2018	2015	very good	very good
CS-FU00406	Fus22	FUSATI	tricinctum	tricinctum	unknown	Barley	Seed	TUM		20.03.2018		very good	very good
CS-FU00407	Fus24	FUSATI	avenaceum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00408	Fus25	FUSATI	tricinctum	tricinctum	unknown		Seed	TUM		20.03.2018		very good	very good
CS-FU00409	Fus26	FUSATI	avenaceum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00410	Fus28	FUSATI	tricinctum	tricinctum	Danemark	Barley	Seed	TUM	Dänemark	20.03.2018	2015	very good	very good
CS-FU00411	Fus29	FUSATI	culmorum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00412	Fus30	FUSATI	tricinctum, avenaceum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00413	Fus31	FUSAPO	Sporotrichoides	poae	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00414	Fus32	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Bayern	20.03.2018	2015	very good	very good
CS-FU00415	Fus33	FUSATI	tricinctum	tricinctum	Great Britain	Barley	Seed	TUM	England	20.03.2018	2015	very good	very good
CS-FU00416	Fus34	GIBBZE	graminearum	graminearum	Germany	Barley	Seed	TUM	Freising	20.03.2018	2015	very good	very good
CS-FU00417	Fus35	GIBBZE	graminearum	graminearum	Germany	Barley	Seed	TUM	Freising	20.03.2018	2015	very good	very good
CS-FU00418	Fus36	FUSATI	tricinctum	tricinctum	unknown		Seed	TUM		20.03.2018		very good	very good
CS-FU00419	Fus37	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00420	Fus38	FUSASP	tricinctum, avenaceum	sporotrichoides	Germany		Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good

CS-FU00421	Fus39	FUSATI	avenaceum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00422	Fus40	FUSAVR	avenaceum		Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	bad	bad
CS-FU00423	Fus41	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00424	Fus42	FUSAPO	poae	poae	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00425	Fus43	FUSATI	Sporotrichoides	tricinctum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00426	Fus44	FUSATI	tricinctum	tricinctum	Germany	Barley	Seed	TUM	Bayern	20.03.2018	2015	very good	very good
CS-FU00427	Fus46	FUSATI	tricinctum, avenaceum	tricinctum	Germany	Barley	Seed	TUM	Bayern	20.03.2018	2015	very good	very good
CS-FU00428	Fus47	GIBBZE	graminearum	graminearum	Germany	Barley	Seed	TUM	Dietlingen	20.03.2018	2015	very good	very good
CS-FU00429	Fus48	GIBBZE	graminearum	graminearum	Germany	Barley	Seed	TUM	Freising	20.03.2018	2015	very good	very good
CS-FU00430	Fus50	GIBBAV	avenaceum	avenaceum	Austria	Barley	Seed	TUM	österreich	20.03.2018	2015	very good	very good
CS-FU00431	Fus51	GIBBAV	avenaceum	avenaceum	Germany	Barley	Seed	TUM	Baden-Württemberg	20.03.2018	2015	very good	very good
CS-FU00432	Fus52	FUSATI	tricinctum	tricinctum	unknown		Seed	TUM		20.03.2018		very good	very good
CS-FU00433	Fus53	FUSAOPO	tricinctum	poae	Germany	Barley	Seed	TUM	Thüringen	20.03.2018	2015	very good	very good
CS-FU00434	1082	GIBBZE	graminearum	graminearum	France	Mais	Seed		Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00435	1083	FUSAPF	graminearum	proliferatum	France	Mais	Seed		Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00436	1084	GIBBZE	graminearum	graminearum	France	Mais	Seed		Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00437	1086	GIBBZE	graminearum	graminearum	France	Mais	Seed		Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00438	1087	FUSACW	graminearum	cerealis	France	Mais	Seed		Goumoens VD	24.05.2018	2006	very good	very good

CS-FU00439	1088	GIBBZE	graminearum	graminearum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00440	1089	FUSAPF	graminearum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00441	1090	GIBBZE	graminearum	graminearum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00442	1151	GIBBZE	graminearum	graminearum	France	Mais	Seed	Baden AG	24.05.2018	2005	very good	very good
CS-FU00443	1152	GIBBZE	graminearum	graminearum	France	Mais	Seed	Baden AG	24.05.2018	2005	very good	very good
CS-FU00444	1153	GIBBZE	graminearum	graminearum	France	Mais	Seed	Baden AG	24.05.2018	2005	very good	very good
CS-FU00445	1154	GIBBZE	graminearum	graminearum	France	Mais	Seed	Baden AG	24.05.2018	2005	very good	very good
CS-FU00446	1155	GIBBZE	graminearum	graminearum	France	Mais	Seed	Baden AG	24.05.2018	2005	very good	very good
CS-FU00447	1156	GIBBZE	graminearum	graminearum	France	Mais	Seed	Baden AG	24.05.2018	2005	very good	very good
CS-FU00448	1133	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00449	1134	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00450	1135	FUSAPF	verticillioides	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00451	1136	FUSAVR	verticillioides	verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00452	1137	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00453	1138	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00454	1139	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00455	1140	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00456	1141	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00457	1142	FUSAPF	verticillioides	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good

CS-FU00458	1143	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00459	1157	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2007	bad	very good
CS-FU00460	1158	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2007	bad	very good
CS-FU00461	1159	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2007	bad	very good
CS-FU00462	1160	FUSAVR	verticillioides	Verticillioides	France	Mais	Seed	Goumoens VD	24.05.2018	2007	bad	very good
CS-FU00463	1109	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00464	1110	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00465	1111	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00466	1112	GIBBFS	proliferatum	subglutinans	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00467	1113	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00468	1114	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00469	1115	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00470	1116	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00471	1117	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00472	1118	FUSAPF	proliferatum	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00473	1122	GIBBFS	subglutinans	subglutinans	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00474	1123	GIBBFS	subglutinans	subglutinans	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00475	1124	GIBBFS	subglutinans	subglutinans	France	Mais	Seed	Goumoe	24.05.2018	2006	very good	very good

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CS-FU00476	1125	GIBBFS	subglutinans	subglutinans	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00477	1126	GIBBFS	subglutinans	subglutinans	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00478	1128	FUSAPF	subglutinans	proliferatum	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00479	1129	GIBBFS	subglutinans	subglutinans	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00480	1130	GIBBFS	subglutinans	subglutinans	France	Mais	Seed	Goumoens VD	24.05.2018	2006	very good	very good
CS-FU00481	1056	FUSACW	Cerealis	cerealis	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00482	1057	FUSACW	Cerealis	cerealis	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00483	1058	FUSACW	Cerealis	cerealis	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00484	1059	FUSACW	Cerealis	cerealis	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00485	1060	FUSACW	Cerealis	cerealis	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00486	1061	FUSATRI	Cerealis	tricinctum	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00487	1062	FUSACU	Cerealis	culmorum	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00488	1063	GIBBZE	Cerealis	graminearum	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00489	1064	FUSACW	Cerealis	cerealis	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00490	1074	GIBBIN	equiseti	equiseti	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00491	1075	FUSAPF	equiseti	proliferatum	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00492	1076	FUSAPF	equiseti	proliferatum	France	Maize	seed	Goumoens VD	16.01.2019	2006	very good	very good

CS-FU00493	1077	GIBBIN	equiseti	equiseti	France	Maize	seed		Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00494	1078	GIBBIN	equiseti	equiseti	France	Maize	seed		Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00495	1079	FUSAPF	equiseti	proliferatum	France	Maize	seed		Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00496	1080	GIBBIN	equiseti	equiseti	France	Maize	seed		Goumoens VD	16.01.2019	2006	very good	very good
CS-FU00497	12FusCH01.02	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00498	12FusCH02.01	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00499	12FusCH02.04	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00500	12FusCH03.03	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00501	12FusCH03.09	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00502	12FusCH07.03	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00503	12FusCH07.05	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00504	12FusCH12.02	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00505	12FusCH12.04	GIBBIN	equiseti	equiseti	Switzerland		seed		Les Barges	21.01.2019	2012	very good	very good
CS-FU00506	12FusF01.04	GIBBIN	equiseti	equiseti	France		seed		67350 Ettendorf	21.01.2019	2012	very good	very good
CS-FU00507	12FusF11.14	GIBBIN	equiseti	equiseti	France		seed		80330 CAGNY	21.01.2019	2012	very good	very good
CS-FU00508	13FusD31.07	GIBBIN	equiseti	equiseti	Germany		seed		49762 Lathen	21.01.2019	2013	very good	very good
CS-FU00509	14FusCH01.08	GIBBIN	equiseti	equiseti	Switzerland		seed		3977 Granges	21.01.2019	2014	very good	very good
CS-FU00510	14FusCH06.06	GIBBIN	equiseti	equiseti	Switzerland		seed		3977 Granges	21.01.2019	2014	very good	very good
CS-FU00511	14FusDE04.04	GIBBIN	equiseti	equiseti	Germany		seed		23936 Harmshagen	21.01.2019	2014	very good	very good
CS-FU00512	15FusDE03.04	GIBBIN	equiseti	equiseti	Germany		seed		35410	21.01.2019	2015	very good	very good

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CS-FU00513	15FusDE18.01	GIBBIN	equiseti	equiseti	Germany	seed	31636 Linsburgn	21.01.2019	2015	very good	very good
CS-FU00514	14FusPl_CH2.01	GIBBIN	equiseti	equiseti	Switzerland	Plants	3977 Granges	21.01.2019	2014	very good	very good
CS-FU00515	14FusPl_CH2.02	GIBBIN	equiseti	equiseti	Switzerland	Plants	3977 Granges	21.01.2019	2014	very good	very good
CS-FU00516	14FusPl_CH2.03	GIBBIN	equiseti	equiseti	Switzerland	Plants	3977 Granges	21.01.2019	2014	very good	very good
CS-FU00517	14FusPl_CH2.04	GIBBIN	equiseti	equiseti	Switzerland	Plants	3977 Granges	21.01.2019	2014	very good	very good
CS-FU00518	14FusPl_DE02.05	GIBBIN	equiseti	equiseti	Germany	Plants	04720 Döbeln	21.01.2019	2014	very good	very good
CS-FU00519	14FusPl_DE05.08	GIBBIN	equiseti	equiseti	Germany	Plants	23936 Harmshagen	21.01.2019	2014	very good	very good
CS-FU00520	14FusPl_DE07.02	GIBBIN	equiseti	equiseti	Germany	Plants	31542 Riepen	21.01.2019	2014	very good	very good
CS-FU00521	14FusPl_DE20.06	GIBBIN	equiseti	equiseti	Germany	Plants	31191 Hannover	21.01.2019	2014	very good	very good
CS-FU00522	14FusPl_DE32.04	GIBBIN	equiseti	equiseti	Germany	Plants	06917 Jessen	21.01.2019	2014	very good	very good
CS-FU00523	11FusPL05	GIBBIN	equiseti	equiseti	Poland	seed	64-000 Koscian	21.01.2019	2011	very good	very good
CS-FU00524	11FusSK05	GIBBIN	equiseti	equiseti	Slovakia	seed	97901 Rimavská Sobota	21.01.2019	2011	very good	very good
CS-FU00525	16FusI06.02	GIBBIN	equiseti	equiseti	Italy	seed	26020 Cremona	21.01.2019	2016	very good	very good
CS-FU00526	16FusI06.07	GIBBIN	equiseti	equiseti	Italy	seed	26020 Cremona	21.01.2019	2016	very good	very good
CS-FU00527	16FusUA01.10	GIBBIN	equiseti	equiseti	Ukraine	seed	9100 Bila Tserkva	21.01.2019	2016	very good	very good
CS-FU00528	16FusUA03.01	GIBBIN	equiseti	equiseti	Ukraine	seed	9100 Bila Tserkva	21.01.2019	2016	very good	very good
CS-FU00529	16FusUA04.08	GIBBIN	equiseti	equiseti	Ukraine	seed	9100 Bila Tserkva	21.01.2019	2016	very good	very good

CS-FU00530	17FusUA02.01	GIBBIN	equiseti	equiseti	Ukraine	seed	Khmelnitskiy	21.01.2019	2017	very good	very good
CS-FU00531	17FusPL03.10	GIBBIN	equiseti	equiseti	Poland	seed	Lany Wielkie	21.01.2019	2017	very good	very good
CS-FU00532	17FusRU01.02	GIBBIN	equiseti	equiseti	Russia	seed	Krasnodar	21.01.2019	2017	very good	very good
CS-FU00533	14FusPl_AT01.03	GIBBIN	equiseti	equiseti	Austria	Plants	2102 Bisamberg	21.01.2019	2014	very good	very good
CS-FU00534	14FusPl_AT01.04	GIBBIN	equiseti	equiseti	Austria	Plants	2102 Bisamberg	21.01.2019	2014	very good	very good
CS-FU00535	14FusPl_CZ01.03	GIBBIN	equiseti	equiseti	Czech Republic	Plants	34401 Straz	21.01.2019	2014	very good	very good
CS-FU00536	14FusPl_DK07.02	GIBBIN	equiseti	equiseti	Denmark	Plants	4100 Ringsted	21.01.2019	2014	very good	very good
CS-FU00537	10FusCZ4.1		cerealis		Czech Republic	Seed		21.01.2019	2014	bad	bad
CS-FU00538	10FusCZ4.2	GIBBIN	cerealis	equiseti	Czech Republic	Seed		21.01.2019	2014	very good	very good
CS-FU00539	11FusD12	FUSACW	cerealis	cerealis	Germany	Seed		21.01.2019	2014	very good	very good

Table 2: Maintenance products during the wheat assays 2017, 2018, 2019

- 2017

No.	Date	Area	Type	Type Description	Ingred. Type	PRODUCT NAME [DSP]	Product Name	
1.	19.10.2016	Trial	FERT	Fertilization	FER	Maintenance Prod Fertilizer [-;UN]	Maintenance Prod Fertilizer	-
2.	22.2.2017	Trial	FERT	Fertilization	FER	Maintenance Prod Fertilizer [-;UN]	Maintenance Prod Fertilizer	-
3.	23.2.2017	Trial	FERT	Fertilization	FER	Maintenance Prod Fertilizer [-;UN]	Maintenance Prod Fertilizer	-
4.	23.2.2017	Trial	FERT	Fertilization	FER	Maintenance Prod Fertilizer [-;UN]	Maintenance Prod Fertilizer	-
5.	30.3.2017	Trial	FERT	Fertilization	FER	Maintenance Prod Fertilizer [-;UN]	Maintenance Prod Fertilizer	-
6.	5.4.2017	Trial	PEST	Pesticide application	AII	Biscaya 240 OD [-;UN]	Biscaya 240 OD	-
7.	5.4.2017	Trial	PEST	Pesticide application	AIF	Legend 250 SC [-;UN]	Legend 250 SC	-
8.	13.4.2017	Trial	PEST	Pesticide application	AIF	Astor 750 EC [-;UN]	Astor 750 EC	-
9.	24.4.2017	Trial	PEST	Pesticide application	AIF	Cyflamid 50 SC [-;UN]	Cyflamid 50 SC	-
10.	10.5.2017	Trial	PEST	Pesticide application	AIF	Amistar Xtra 280 SC [-;UN]	Amistar Xtra 280 SC	-
11.	10.5.2017	Trial	PEST	Pesticide application	AII	Biscaya 240 OD [-;UN]	Biscaya 240 OD	-
12.	17.5.2017	Trial	FERT	Fertilization	FER	Maintenance Prod Fertilizer [-;UN]	Maintenance Prod Fertilizer	-
13.	17.5.2017	Trial	PEST	Pesticide application	AIF	Opus TOP 334 SE [-;UN]	Opus TOP 334 SE	-
14.	2.6.2017	Trial	PEST	Pesticide application	AII	Biscaya 240 OD [-;UN]	Biscaya 240 OD	-

- 2018

No.	Date	Type	Ingred. Type	Product Name	Form Variant	Country	Form Type	Form Conc	Form Unit	Rate	Unit	Comment
1.	17.10.2017	FERT	FER	Maintenance Prod Fertilizer	-	UN				250.0	KGPR/HA	Landor Triphoska
2.	28.2.2018	FERT	FER	Maintenance Prod Fertilizer	-	UN				150.0	KGPR/HA	Landor Triphoska
3.	28.2.2018	FERT	FER	Maintenance Prod Fertilizer	-	UN				250.0	KGPR/HA	Landor Mg-Ammonsalpeter+S
4.	1.3.2018	DPOG	FER	Maintenance Prod Fertilizer	-	UN				250.0	KGPR/HA	Dolomit Magnesiumkalk
5.	4.4.2018	FERT	FER	Maintenance Prod Fertilizer	-	UN				200.0	KGPR/HA	Landor Mg-Ammonsalpeter+S
6.	19.4.2018	PEST	AIF	Amistar Xtra 280 SC	-	UN	SC	280	GA/L	1.0	LPR/HA	
7.	27.4.2018	PEST	AIF	Astor 750 EC	-	UN	EC	750	GA/L	0.75	LPR/HA	
8.	4.5.2018	PEST	AII	Biscaya 240 OD	-	UN	OD	240	GA/L	0.3	LPR/HA	
9.	4.5.2018	PEST	AIF	Amistar Xtra 280 SC	-	UN	SC	280	GA/L	1.0	LPR/HA	
10.	14.5.2018	PEST	AIF	Opus TOP 334 SE	-	UN	SE	334	GA/L	1.5	LPR/HA	
11.	24.5.2018	PEST	AII	Biscaya 240 OD	-	UN	OD	240	GA/L	0.3	LPR/HA	

- 2019

No.	Date	Type	Ingred. Type	Product Name	Form Variant	Country	Form Type	Form Conc	Form Unit	Rate	Unit	Comment
1.	25.3.2019	WEED	AIH	Archipel 15 OD	-	CH	OD	15	GA/L	1.64	LPR/HA	
2.	26.2.2019	FERT	FER	Maintenance Prod Fertilizer	-	UN				250.0	KGPR/HA	Landor Mg-Ammonsalpeter+S
3.	26.2.2019	FERT	FER	Maintenance Prod Fertilizer	-	UN				400.0	KGPR/HA	Landor PK
4.	26.3.2019	FERT	FER	Maintenance Prod Fertilizer	-	UN				400.0	KGPR/HA	Landor Ammonsalpeter
5.	15.4.2019	PEST	AII	Biscaya 240 OD	-	UN	OD	240	GA/L	0.3	LPR/HA	
6.	23.4.2019	FERT	FER	Maintenance Prod Fertilizer	-	UN				100.0	KGPR/HA	Landor Ammonsalpeter
7.	16.4.2019	PEST	AIF	Amistar Xtra 280 SC	-	UN	SC	280	GA/L	1.0	LPR/HA	
8.	16.4.2019	PEST	AIR	Moddus 250 ME	-	UN	ME	250	GA/L	0.4	LPR/HA	
9.	30.4.2019	PEST	AIR	Ethephon	-	UN				0.5	LPR/HA	Ethefon S
10.	7.6.2019	PEST	AIF	Astor 750 EC	-	UN	EC	750	GA/L	0.75	LPR/HA	
11.	16.5.2019	PEST	AIF	Amistar Xtra 280 SC	-	UN	SC	280	GA/L	1.0	LPR/HA	
12.	16.5.2019	PEST	AIF	Bravo 500	-	UN	SC	500	GA/L	0.5	LPR/HA	

Table 3: Maintenance products during the corn assays 2017, 2018, 2019

- 2017

No.	Date	Type	Ingred. Type	Product Name	Form Variant	Country	Rate	Unit	Comment
1.	24.4.2017	FERT	FER	Maintenance Prod Fertilizer	-	UN	500.0	KGPR/HA	Triphoska
2.	24.4.2017	FERT	FER	Maintenance Prod Fertilizer	-	UN	350.0	KGPR/HA	Kali 60
3.	5.5.2017	FERT	FER	Maintenance Prod Fertilizer	-	UN	100.0	KGPR/HA	DAP 18.46.0 Diammonphosphat
4.	27.6.2017	FERT	FER	Maintenance Prod Fertilizer	-	UN	200.0	KGPR/HA	Harnstoff 46% N

- 2018

No.	Date	Type	Ingred. Type	Product Name	Form Variant	Country	Rate	Unit	Comment
1.	1.5.2018	FERT	FER	Maintenance Prod Fertilizer	-	UN	500.0	KGPR/HA	Triphoska
2.	1.5.2018	FERT	FER	Maintenance Prod Fertilizer	-	UN	100.0	KGPR/HA	DAP 18.46.0 Diammonphosphat
3.	12.6.2018	FERT	FER	Maintenance Prod Fertilizer	-	UN	200.0	KGPR/HA	Harnstoff 46% N

- 2019

No.	Date	Type	Ingred. Type	Product Name	Form Variant	Country	Form Type	Form Conc	Form Unit	Rate	Unit
1.	25.4.2019	MAIN	AIH	Lumax 537.5 SE	-	UN	SE	537.5	GA/L	4.0	LPR/HA
2.	1.7.2019	MAIN	AII	Karate Zeon 100 CS	-	AR	CS	100	GA/L	0.075	GPR/HA
3.	3.7.2019	MAIN	AIF	Amistar 250 SC	-	UN	SC	250	GA/L	1.0	LPR/HA
4.	3.7.2019	MAIN	AII	Coragen 200 SC	-	UN	SC	200	GA/L	4.0	GPR/HA

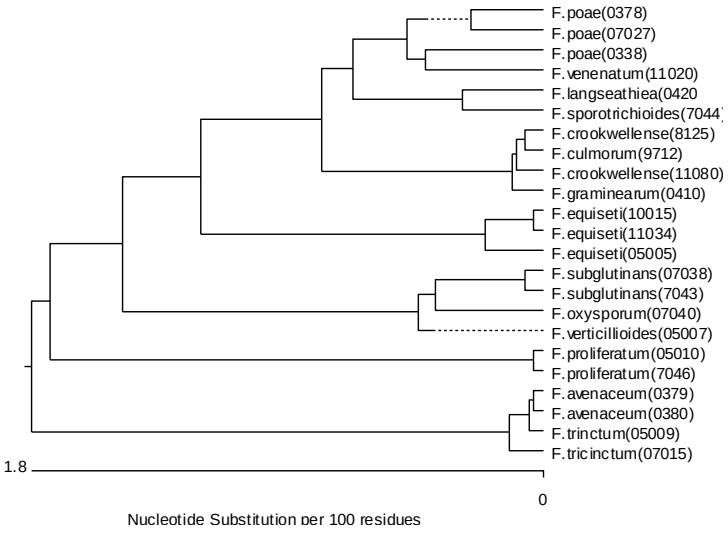
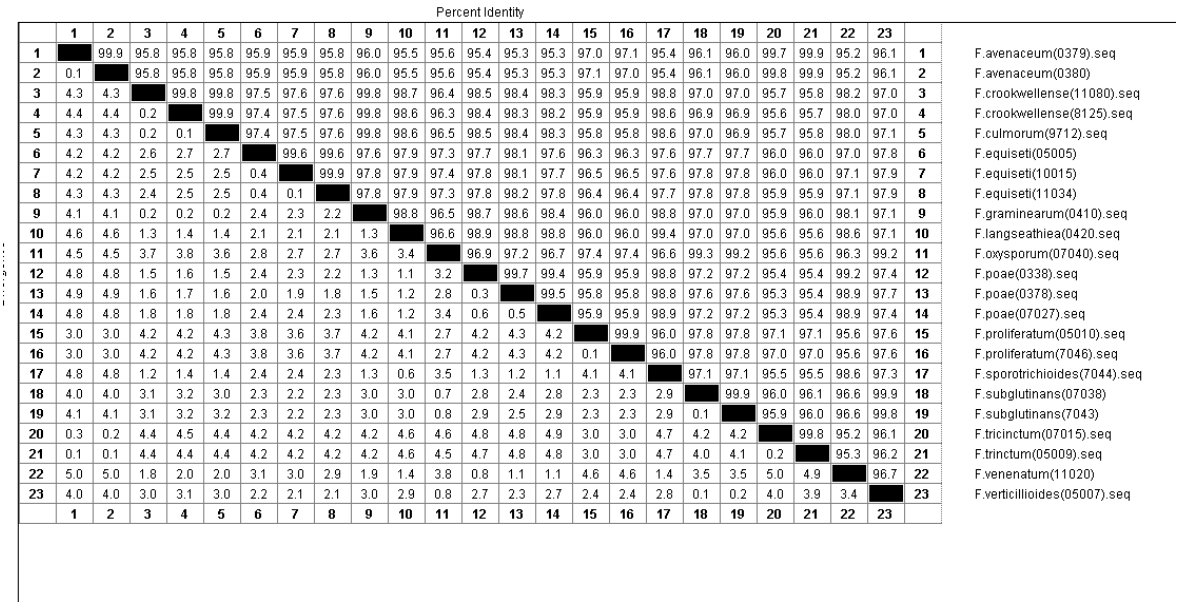


Figure 1: Phylogeny of Fusarium *reference sequences using MegAlign software (Walder et al., 2017)*



Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
1		99.9	95.8	95.8	95.8	95.9	95.9	95.8	96.0	95.5	95.6	95.4	95.3	95.3	97.0	97.1	95.4	96.1	96.0	99.7	99.9	95.2	96.1	1	F.avenaceum(0379).seq
2	0.1		95.8	95.8	95.8	95.9	95.9	95.8	96.0	95.5	95.6	95.4	95.3	95.3	97.1	97.0	95.4	96.1	96.0	99.8	99.9	95.2	96.1	2	F.avenaceum(0380)
3	4.3	4.3		99.8	99.8	97.5	97.6	97.6	99.8	98.7	96.4	98.5	98.4	98.3	95.9	95.9	98.8	97.0	97.0	95.7	95.8	98.2	97.0	3	F.crookwellense(11080).seq
4	4.4	4.4	0.2		99.9	97.4	97.5	97.6	99.8	98.6	96.3	98.4	98.3	98.2	95.9	95.9	98.6	96.9	96.9	95.6	95.7	98.0	97.0	4	F.crookwellense(8125).seq
5	4.3	4.3	0.2	0.1		97.4	97.5	97.6	99.8	98.6	96.5	98.5	98.4	98.3	95.8	95.8	98.6	97.0	96.9	95.7	95.8	98.0	97.1	5	F.culmorum(9712).seq
6	4.2	4.2	2.6	2.7	2.7		99.6	99.6	97.6	97.9	97.3	97.7	98.1	97.6	96.3	96.3	97.6	97.7	97.7	96.0	96.0	97.0	97.8	6	F.equiseti(05005)
7	4.2	4.2	2.5	2.5	2.5	0.4		99.9	97.8	97.9	97.4	97.8	98.1	97.7	96.5	96.5	97.6	97.8	97.8	96.0	96.0	97.1	97.9	7	F.equiseti(10015)
8	4.3	4.3	2.4	2.5	2.5	0.4	0.1		97.8	97.9	97.3	97.8	98.2	97.8	96.4	96.4	97.7	97.8	97.8	95.9	95.9	97.1	97.9	8	F.equiseti(11034)
9	4.1	4.1	0.2	0.2	0.2	2.4	2.3	2.2		98.8	96.5	98.7	98.6	98.4	96.0	96.0	98.8	97.0	97.0	95.9	96.0	98.1	97.1	9	F.graminearum(0410).seq
10	4.6	4.6	1.3	1.4	1.4	2.1	2.1	2.1	1.3		96.6	98.9	98.8	98.8	96.0	96.0	99.4	97.0	97.0	95.6	95.6	98.6	97.1	10	F.langseathiea(0420.seq
11	4.5	4.5	3.7	3.8	3.6	2.8	2.7	2.7	3.6	3.4		96.9	97.2	96.7	97.4	97.4	96.6	99.3	99.2	95.6	95.6	96.3	99.2	11	F.oxysporum(07040).seq
12	4.8	4.8	1.5	1.6	1.5	2.4	2.3	2.2	1.3	1.1	3.2		99.7	99.4	95.9	95.9	98.8	97.2	97.2	95.4	95.4	99.2	97.4	12	F.poae(0338).seq
13	4.9	4.9	1.6	1.7	1.6	2.0	1.9	1.8	1.5	1.2	2.8	0.3		99.5	95.8	95.8	98.8	97.6	97.6	95.3	95.4	98.9	97.7	13	F.poae(0378).seq
14	4.8	4.8	1.8	1.8	1.8	2.4	2.4	2.3	1.6	1.2	3.4	0.6	0.5		95.9	95.9	98.9	97.2	97.2	95.3	95.4	98.9	97.4	14	F.poae(07027).seq
15	3.0	3.0	4.2	4.2	4.3	3.8	3.6	3.7	4.2	4.1	2.7	4.2	4.3	4.2		99.9	96.0	97.8	97.8	97.1	97.1	95.6	97.6	15	F.proliferatum(05010).seq
16	3.0	3.0	4.2	4.2	4.3	3.8	3.6	3.7	4.2	4.1	2.7	4.2	4.3	4.2	0.1		96.0	97.8	97.8	97.0	97.0	95.6	97.6	16	F.proliferatum(7046).seq
17	4.8	4.8	1.2	1.4	1.4	2.4	2.4	2.3	1.3	0.6	3.5	1.3	1.2	1.1	4.1	4.1		97.1	97.1	95.5	95.5	98.6	97.3	17	F.sporotrichioides(7044).seq
18	4.0	4.0	3.1	3.2	3.0	2.3	2.2	2.3	3.0	3.0	0.7	2.8	2.4	2.8	2.3	2.3	2.9		99.9	96.0	96.1	96.6	99.9	18	F.subglutinans(07038)
19	4.1	4.1	3.1	3.2	3.2	2.3	2.2	2.3	3.0	3.0	0.8	2.9	2.5	2.9	2.3	2.3	2.9	0.1		95.9	96.0	96.6	99.8	19	F.subglutinans(7043)
20	0.3	0.2	4.4	4.5	4.4	4.2	4.2	4.2	4.2	4.6	4.6	4.8	4.8	4.9	3.0	3.0	4.7	4.2	4.2		99.8	95.2	96.1	20	F.tricinctum(07015).seq
21	0.1	0.1	4.4	4.4	4.4	4.2	4.2	4.2	4.2	4.6	4.5	4.7	4.8	4.8	3.0	3.0	4.7	4.0	4.1	0.2		95.3	96.2	21	F.trinctum(05009).seq
22	5.0	5.0	1.8	2.0	2.0	3.1	3.0	2.9	1.9	1.4	3.8	0.8	1.1	1.1	4.6	4.6	1.4	3.5	3.5	5.0	4.9		96.7	22	F.venenatum(11020)
23	4.0	4.0	3.0	3.1	3.0	2.2	2.1	2.1	3.0	2.9	0.8	2.7	2.3	2.7	2.4	2.4	2.8	0.1	0.2	4.0	3.9	3.4		23	F.verticillioides(05007).seq
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		

Figure 2: Percentage of identity between each species used as reference in the ITS identification

SDHB

>JX869230.1 *F. graminearum* strain 38995 mitochondrial succinate dehydrogenase subunit B (SdhB) gene, complete cds; nuclear gene for mitochondrial product

>XM_018895312.1 *Fusarium verticillioides* 7600 succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial (FVEG_06879), mRNA

SDHC

>JX869216.1 *F. graminearum* strain 37308 mitochondrial succinate dehydrogenase subunit C (SdhC) gene, partial cds; nuclear gene for mitochondrial product

>XM_018895081.1 *Fusarium verticillioides* 7600 hypothetical protein (FVEG_06680), mRNA

SDHD

>JX869209.1 *F. graminearum* strain 37308 mitochondrial succinate dehydrogenase subunit D (SdhD) gene, partial cds; nuclear gene for mitochondrial product

Figure 3: NCBI sequences sdh subunits

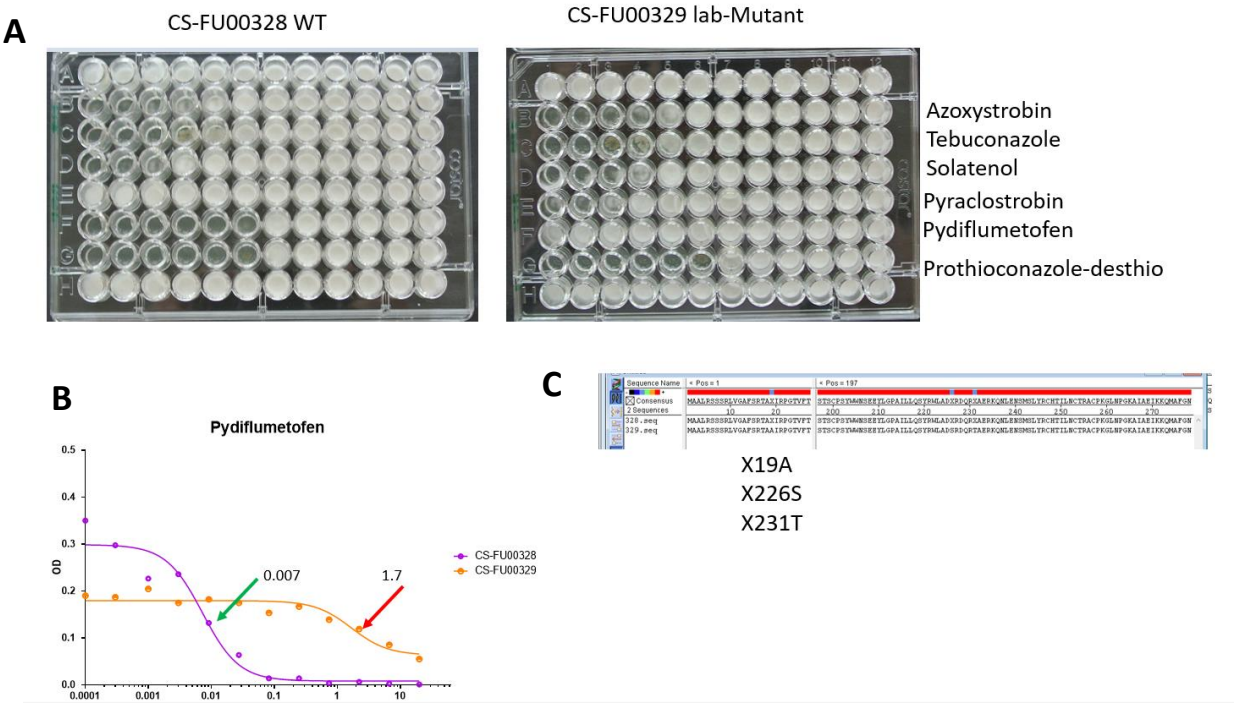


Figure 4: A. 96 well plate in vitro *assay with* F. graminearum *WT and sdh mutant. B. EC50 curves of WT and mutant to Pydiflumetofen. C. Protein alignment of the WT and mutant*

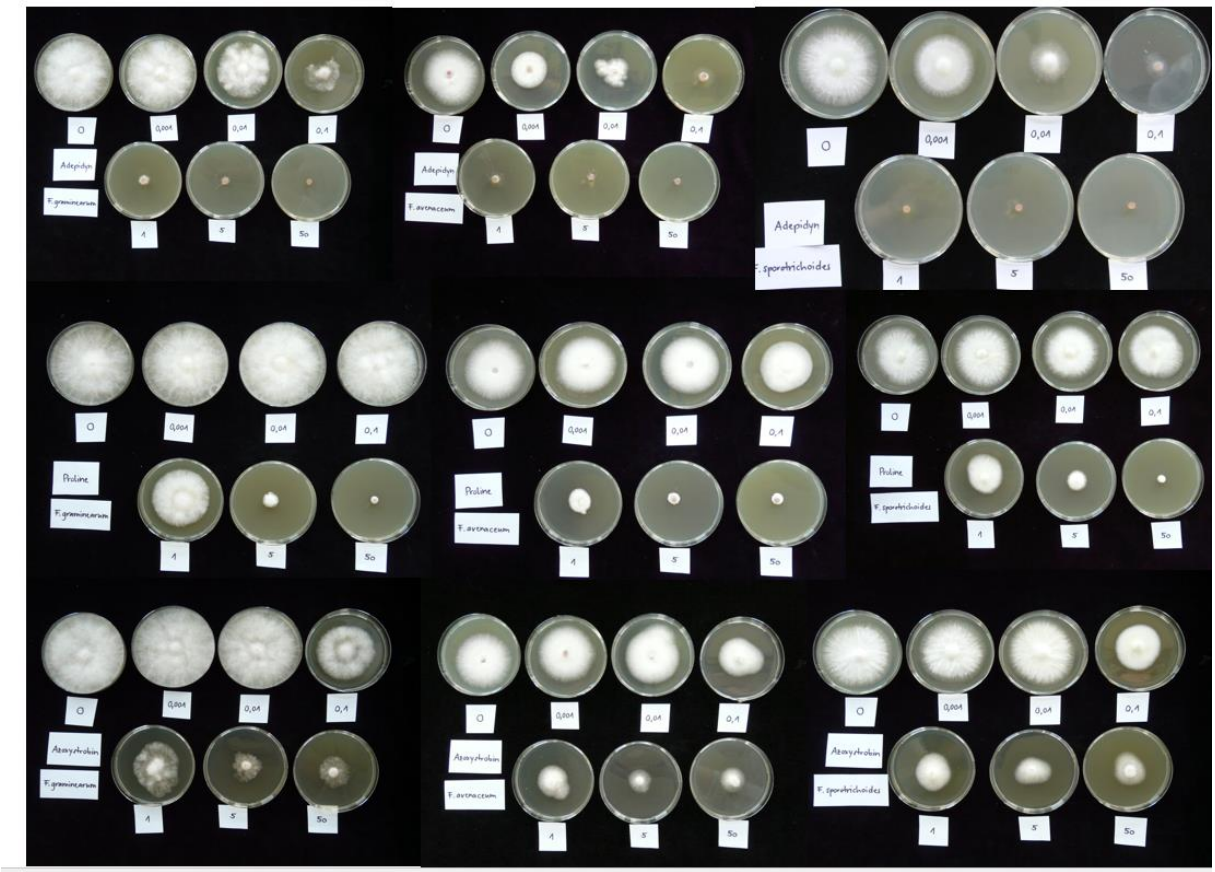


Figure 5: in vitro *fungicide assay on petri dishes with fungicide concentrations from 0 to 50 ppm. First column* is F. graminearum*, second column* F. avenaceum*, third column is* F. sporotrichioides. *First row is with pydiflumetofen, second is prothioconazole-desthio and third row is azoxystrobin.*